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Past, present and future: Geographic and temporal variation in a fig–fig wasp mutualism

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Past, present and future: Geographic and temporal variation in a fig–fig wasp mutualism

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This dissertation is dedicated to Daniel Châtellier,

loving uncle that will remain in my memories as one of the nicest persons I have ever known.

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Six years separate my first arrival at Iowa State University, late 2012, from now, as I am finishing writing this dissertation. A time during which I first arrived a stranger, to nowadays where I feel integrally part of Ames's community, more loaded in knowledge, friends, skills, connections, culture and memories. My journey in both the United States of America and the United Mexican States to obtain a doctoral degree was accompanied by people which supported in many ways to the completion of this dissertation.

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ABSTRACT

All living organisms interact with different degrees of interdependence and in ways that are integral to their ecology and evolution. Of the many forms of species interaction, mutualism is one in which species reciprocally obtain benefits from their interactions. Because mutualism is ubiquitous in nature, mutualists are commonly associated with a broader community of species whose interactions vary across a mutualist–antagonist spectrum and in space and time, and so they play a broadly important role in ecosystem function and species evolution. Flowering plants are widely distributed, typically primary producers, and thus foundational ecosystem elements. They are also ubiquitously associated with a diverse assemblage of insects, including diffuse to species-specific associations with pollinators (mutualists) and herbivores and seed predators (antagonists), which are interactions that effect the reproductive fitness of both plants and insects. The main goal of this dissertation is to investigate the effects of past and current environmental variation on the dynamics of species involved in obligate plant-insect interactions, and to model this dynamic in the context of future climate scenarios. This goal was addressed studying a fig–fig wasp system as a biological model. Fig trees (*Ficus*, family Moraceae) are a well-known example of obligate symbiosis in which the plants serve as hosts to pollinator and non-pollinator fig wasps and other insects whose larva develop within fig fruits. *Ficus petiolaris* is a rock-strangler fig tree, endemic to Mexico, and hosts nine species of chalcidoid fig wasps: one pollinator plus eight species of non-pollinators that are antagonistic to the plant, pollinator or both. In addition, *F. petiolaris* fruit (including developing seeds and wasps) are subject to predation by a species of a lepidopteran larva. Past climatic fluctuations and the geological history of the region are factors that have influenced the co-distribution of these species, a history that can be revealed through the analysis of their contemporary genetic structure. Using

single nucleotide polymorphism (SNP) molecular data, population genetics, phylogenetics, and species distribution modeling, I investigated how the geology, geography and historical climate in western Mexico shaped the genetic landscape of *F. petiolaris,* revealing that northern range limits of *F. petiolaris* shifted to the south during the late Pleistocene, with subsequent range expansion resulting in a contemporary contact zone in coastal northwestern Mexico between previously isolated populations in Baja California and mainland Mexico. I then investigated present day associations between biotic and abiotic ecological variables and local and landscape-level dynamics in fig wasp community composition and lepidopteran fruit predation. The results indicate that variation in *F. petiolaris* reproductive phenology and tree density differentially influence the relative proportions of pollinating and parasitic wasps, as well as the rate of damage cause by the lepidopteran larvae. Furthermore, I found that increasing local temperature and precipitation strongly benefit pollinator reproductive success, and hence pollen dispersal, at the expense of non-pollinator production. Finally, global climate change scenarios in Mexico predict substantial near-future geographical changes in temperature and rainfall. Using future climate modeling, I projected the distribution of *F. petiolaris* and predicted changes in pollinator versus non-pollinator reproductive success to assess implications of climate change for the fig–fig wasp mutualism. Because rapid, human-mediated global environmental change is threatening biodiversity, it is crucial to understand the effect of spatial and temporal environmental variation on species interactions and their consequences. Projections of the *F. petiolaris* system indicate that near-term climate change has the potential to disadvantage the mutualism by decreasing pollinator reproduction success over an expanded geographical area. This dissertation provides new insight into the fig–fig wasp symbiosis and its relationship with its past and current environment, and present for the first time a joint, climate-based projection of a fig's

geographical distribution, wasp community composition, and mutualism dynamics into the future.

CHAPTER 1. GENERAL INTRODUCTION

1. Living organisms in complex environments

1.1 A world of interspecific interactions

All biological forms living on Earth interact with the physical (abiotic) properties of the landscapes in which they occur, as well as with other living organisms. Defined as the biosphere, the ensemble of all the living species and the ecosystems they constitute represents the very dynamic layer encompassed within the geosphere, the lithosphere, the hydrosphere and the atmosphere, and defines in large part the distinctiveness of our fascinating Blue Planet. Although it is not scientifically understood yet how life originated on Earth, there is evidence highlighting changes in the biosphere during the long history of our planet. The oldest existing organisms to date are conical stromatolites (3.45–3.7 Gyr, Allwood et al., 2006; Nutman et al., 2016; but see Allwood et al., 2018), which are interpreted as colonies of microorganisms. They suggest that in the earliest stages of life, living organisms were already interacting with one another. During the exceptional 4.57 Gyr history of Earth, life has experienced increased diversification by speciation from a single uncertain origin through millions of years of evolution (Darwin, 1859). Life was first likely to be mostly aquatic before the colonization of land by living organisms, possibly during the early Paleozoic (Gray & Shear, 1992). However, life on Earth has also experienced dramatic losses in species diversity, most notably during five mass extinctions (Raup, 1986). That we are today able to appreciate and explore the vast diversity of extant species, it is because these are the products of the long evolutionary history of life on Earth, which we are part of. Life is found in most parts of the globe, but unfortunately, it is currently under threat. Indeed, recent evidence supports an ongoing human-driven sixth great extinction, putting numerous species at risk (Barnosky et al., 2011; Ceballos et al., 2017). Even if biotic

recovery is expected within hundreds of thousands to a few millions of years after mass extinctions (Erwin, 2001), rapid losses in biodiversity are of growing concern for the future of mankind.

Today, one can hardly imagine a species that lives isolated from others. Hot springs are extreme habitats in which we can find highly specialized species adapted to very high temperature and even there, different species compete (Brock, 1967). The deep, dark and cold seas were considered as unsuitable for life until the end of the 18th century, however deoxyribonucleic acid (DNA) analyses from deep sea water columns have revealed a large microflora diversity, suggesting that microscopic biodiversity is even larger than estimated (Sogin et al., 2006). Everywhere on the globe, one who is searching for living organisms will probably find them, and we fail to find a species that lives completely isolated from others. Consequently, every species on Earth interacts with others, and species interactions are at the core of many ecosystem properties and processes, such as nutrient cycling and food webs (Loreau et al., 2001; Cardinale et al., 2002; Wilson et al., 2009). As a result, the study of species interactions constitutes one of the most important subdisciplines of the field of ecology and it is essential that these interactions be taken into consideration in order to understand the biology of any living organism.

Ecological communities are defined by populations of at least two species that interact directly or indirectly in a defined geographic space (Agrawal et al., 2007) and these communities comprise the interacting biotic components of ecosystems. The effects of one species one on another can be positive (+) if it increases the fitness of the species it interacts with, negative (–) if this interaction decreases this fitness or neutral (0) if it does not affect the fitness of the interacting species. Within ecosystems, defining the exact nature of interactions between species

can be laborious because, first, it is hard to quantify the net effect of one species on another and, second, these interactions can vary depending on the evolutionary and environmental context in which they occur (Chamberlain et al., 2014). Nonetheless, interactions between species can be described being a beneficial (+), a disadvantageous (–) or neutral (0) in effect on the other species' reproductive fitness. These interactions can then be categorized according to reciprocal effects: predation (+,–) which includes also parasitism and herbivory; mutualism (+,+); neutralism (0,0); commensalism (+,0); amensalism (–,0); and competition (–,–). However, it has been argued that this simple classification reflects only extremes in a whole spectrum of interactions which includes various quantitative effects between two interacting species (Figure 1, Bronstein, 2015). Interactions between a pair of species can also be obligate or facultative, specific or diffuse, symbiotic or non-symbiotic, and symmetric or asymmetric. But species are not interacting only in pairs; they are part of a multipartite complex in which they affect others to different degrees and different frequencies, and with varying levels of dependency. Taking this complexity into account led scientists to develop the concept of food webs in which prey–predator interactions were initially represented (Pimm et al., 1991). Later, other types of species interactions were incorporated into ecological networks (Montoya et al., 2006). Furthermore, the net effect and outcomes of interactions between species is not fixed; they can vary within populations or be influenced by environmental context (Thompson, 1988). Thus, the study of community complexity has been foundational to the emergence of community ecology, a large subfield of ecology that aims to precisely describe species interactions within communities and factors that shape their community structure, distribution, abundance and diversity.

1.2. Evolutionary aspects

Evolution of species involves changes of allele frequencies within and between biological populations over time. Evolution within species is only possible given genetic variation between individuals and the heritable characteristics of DNA. Variation arises from an essential mechanism in evolution: genetic mutation. Genetic variation created by mutations generate novel alleles, which frequencies change through space and time due to other key mechanisms of evolution (i.e., selection, migration, genetic drift and hitchhiking and nonrandom matting). Within these evolutionary drivers, natural selection is the differential survival of individuals based on their variation in phenotypes (Darwin, 1859), which allows individuals with adaptive traits to accommodate to their environment.

Any factor in the environment affecting individuals' fitness, can induce an evolutionary response at the population or species level. When interacting species are affecting each other (i.e., any type of interaction but neutralism, Figure 1.), reciprocal evolutionary response is expected by natural selection. The concept of coevolution was already mentioned in Darwin's foundational book but not explicitly (e.g., "coadaptation", 1859). Since then, many authors have invoked coevolution as an important driver of diversification (Thompson, 2005; Jablonski, 2008), even if these affirmations have been more nuanced since (Hembry et al., 2014). Coevolution is often seen as an arms race. Interacting species tend to evolve adaptive traits involved in the interaction which increase their own net fitness at the expense of the fitness of the other species. This is particularly the case in antagonistic interactions such as prey–predator in which a predator evolving a character which increases its fitness but reduces the one of the prey, thus changing the evolutionary pressure on its prey, which in turn gives rise to new adaptive traits in the prey in response (Dawkins & Krebs, 1979). But coevolution is not confined

only to coevolving traits in highly specialized interactions such as specific prey–predator systems, coevolution includes any sort of reciprocal adaptive responses of interacting species (Thompson, 1994). The degree of coevolution among interacting species depends on the type of interaction involved, its characteristics and the context in which they occur, and remain relatively unexplored even today (Bronstein et al., 2004).

Coevolution should be particularly important in species with strong interaction dependency, i.e., in species which share an important part of their life with another. In symbiotic interactions, hosts and symbionts live in a tight long-term relationship in which species may highly influence one another. Some interactions are obligate, which means a one species cannot live without another. In symbiotic and/or obligate interactions, the dependence level of these interactions can have important demographic consequences because obligate species' life histories can track each other: where one species is found, the other should be found too. Obligate parasites, for example, can occur only within the range of their host, but hosts can exist outside their parasite's range. Obligate mutualists, however, should be distributed together because of the strong dependence on each other. The last example illustrates how tight interactions can influence each other's spatial distribution: the range of each obligate mutualist is limited not only by its own dispersal abilities, but also by that of its symbiont (in facultative mutualisms a species can exist outside of its mutualist's range, but its fitness is reduced). The degree to which species interactions impact species' ranges and community assemblages will however rely on the level of dependency and characteristics of the interactions (Wisz et al., 2013; Godsoe et al., 2017). Interacting species usually have very different life history traits and evolutionary responses to the environment, which can result in different demographic processes. However, tight interactions can shape similar demographic processes between the interacting

species. Extreme but common examples in nature are the cases of intracellular endosymbionts (i.e., two species of symbionts living inside the cells of their host), which develop extreme dependence on their host, and are vertically transmitted together to the next generation (Ewald, 1987; Moran, 2007). In these cases, both species display identical evolutionary histories (i.e., matching phylogenies), (Hafner & Nadler, 1988; Moran et al., 1993; Chen et al., 1999; Sauer et al., 2000). This contrasts with facultative diffuse interactions, as in the case of generalist pollinators and plants, for which different evolutionary histories are expected (Yokoyama, 1994). However, congruent evolutionary histories can also be driven by similar responses to historical environmental fluctuations (Ricklefs & Schluter, 1993). Thus, within communities, species are associated in various ways, influencing each other's ecology and evolution differentially depending on the closeness of their interactions.

1.3. Temporal and geographic variations of the environment and consequences on species interactions

The environment is complex and varies in both space and time, and species distributed in this environment have variable patterns, both in their diversity (Rosenzweig, 1995) and the distribution of genotypes and phenotypes (Avise, 2000). This variability results from past demographic and evolutionary histories and responses to environmental variation that shaped their contemporary distribution and structure. Thus, there is variation in the phenotypic distribution of individuals within species and the environmental context in which they exist. This observation led scientists to reconsider species interactions: they were initially studied as a single population of one species interacting with a single population of another, but later considered as geographically variable in interaction sign (i.e., 0, + or −), magnitude, and resulting outcome

depending on the environmental context (Thompson, 1988; 1994; 2005; Bronstein, 1994a; Travis, 1996; Holland & DeAngelis, 2009; Chamberlain, Bronstein & Rudgers, 2014).

Thompson (1994; 2005) explored this concept with an evolutionary perspective in his geographic mosaic of coevolution theory. His view is that species are composed of sets of populations, each of which occurs in different environmental contexts. These populations interact with another species' populations, and natural selection in both species favors local adaptation and coevolution. Local adaptation at fine geographic scales may structure populations into genetically distinct groups (Mopper, 1996). The resulting geographic pattern of genetic structure and the level at which local adaptation can occur will, however, depend on the distribution of individuals and dispersal between populations (Thompson & Cunningham, 2002). Species do not coevolve, populations do. And they do so in geographical mosaics, which represent the geographical context in which they occur. For populations that coevolve, a certain degree of congruent genetic structure between interacting species is expected (Anderson et al., 2004).

Contemporary species' genetic and phenotypic distributions have been shaped by ancient environmental variation. Investigating past effects of environmental changes is challenging because it requires knowing the climatic and geological history of the area in which species are distributed, and the evolutionary history of species. This is possible, however, thanks to the inference of past climatic and geological events and the interpretation of the distribution of genetic variation within species. Study of the link between historical events and contemporary distribution of species has long time taken place in biology with the field of historical biogeography. The younger field of phylogeography introduced by Avise et al. (1987) adds to the an intra-specific perspective. With the help of modern techniques of DNA sequencing, the

use of population genetics and phylogenetics in a geographical context allows insights into the contemporary, shallow and deep time evolutionary history of species.

2. Mutualisms

2.1. Mutualisms: reciprocal beneficial interactions between species

Among the diversity of species interactions, a mutualism is the case where species obtain reciprocal benefits from their interaction. The terms “cooperation”, “altruism” or “reciprocal altruism” have also been utilized to describe these types of interactions (“altruism” is, however, avoided as it implies the sacrifice of an individual to the benefit to another, and leads to confusion; Bronstein, 1994a) . The (+) in the schematic representation of mutualisms should however be interpreted with caution, as it represents the net benefit that species obtain by association with another. Indeed, most of the benefits received from another organism comes at a cost to the provider (Bronstein, 2001a). The cost, however, is exceeded by the benefit, and when both organisms reap greater benefits than costs from their association, it is a mutualism. Such associations are ubiquitous in nature (Boucher et al., 1982; Margulis & Fester, 1991) and Janzen even stated in 1985 that mutualisms “are the most omnipresent of any organism–to–organism interaction”. But curiously, mutualism received little attention in the scientific literature before the last third of the 20th century (Bronstein, 1994b). Thanks to dedicated scientists, mutualisms are now recognized as diverse, abundant, ecologically and evolutionary important interactions, and have attained their rightful place in ecology textbooks. The latest effort to summarize current knowledge on mutualisms is a book edited by Bronstein and recently published (2015). Nevertheless, mutualisms are still underappreciated today. For example, when one invokes microorganisms in a human context, one often thinks only about human pathogens, forgetting the

many microorganisms that have contributed and still contribute positively to our lives and societies (Selosse, 2017).

Evidence of the importance of mutualistic association can be traced back to the origin of essential organelles: chloroplasts and mitochondria (Margulis, 1981; Gray, 1989). The endosymbiotic theory holds that these organelles were once free-living prokaryotes and became organelles of eukaryotic cells. Their biochemical and physiological properties, and their reduced individual genomes support this hypothesis. Today, the endosymbiotic theory is the dominant explanation for the origin of organelles in eukaryotes (Zimorski et al., 2014). Thus, mutualisms are everywhere. One who looks at the earth from space sees green patches corresponding to large forests. But as chlorophyll is produced in chloroplasts only, what one really sees are billions of biomolecules synthetized by long-time mutualistic endosymbionts in plants.

Humans have long benefited from mutualistic partners, first with microorganism communities, most obviously in the intestinal lumen, but also on skin and mucosal surfaces (Bäckhed et al., 2005; Dethlefsen et al., 2007). Different forms of mutualistic relationships can be observed between humans and their domesticated plants and animals (Zeder, 2015). Most important, mutualistic interactions have important roles in ecosystem function. Flowering plants (Angiosperms), which are the major primary producers of food and the foundation of many terrestrial habitats, interact most of the time with insect pollinators with which they share an impressive coevolutionary history (Lunau, 2004; Crepet & Niklas 2009). It is estimated that 85% of the angiosperms are pollinated by animals (Ollerton et al., 2011). Plant seeds are also often dispersed by various animals which receive food in return. Mutualisms are also extremely common in soil, forming positive associations with plants (e.g., mycorrhizae and *Rhizobium*; Denison & Kiers, 2011), as well as other soil organisms (Lavelle et al., 1995), and have

important functions in soils as, for example, in nutrient cycling. In the oceans, coral reefs represent often very large structures that host a large diversity of aquatic organisms, and are made of calcium carbonate skeletons from cnidarians forming an endosymbiotic relationship with dinoflagellate algae. It is the fragility of this symbiosis under environmental changes that is leading to the current loss of major coral reefs (Hoegh-Guldberg, 1999; Hoegh-Guldberg et al., 2017). These few examples represent a fraction of the large list of mutualisms in nature many of which are essential for ecosystem functioning.

Symbiosis refers to a long-term tight relationship between organisms (De Bary, 1879; but see Douglas for other uses of the term in the literature, 2010). Symbiotic mutualisms, where both species share persistently at least one part of their life cycle, are ubiquitous and have been the foundation of evolutionary transitions (Maynard Smith & Szathmäry,1995). Microbial endosymbiosis (i.e., symbiont inside its host's cell) is not only at the origin of organelles in eukaryotes but is also found across a multitude of taxa with different level of dependency (i.e., different transition mode and degree of dependence) between hosts and symbionts (Fisher et al., 2017). Symbiotic mutualisms are not always endosymbiotic: tight mutualisms can occur when organisms interact at the surface of others, like animal gut bacteria or mycorrhizae. In these cases, symbionts do not penetrate inside their host cells but live on the surface of their host, creating a surface with their host that will be the place where beneficial exchanges occur. On the other hand, non-symbiotic mutualisms refer to reciprocal interactions with benefits that do not involve long-term interactions. We say then that both species are free living. Obvious examples of non-symbiotic mutualisms are many plant–pollinator interactions where pollinators get the benefit of nutritive resources (nectar and/or pollen) provided by the plant against the service of pollination. Like symbiosis, obligation (when mutualists require their mutualistic partner to live

and reproduce) is also a characteristic of some mutualisms. Obligate mutualisms are particularly interesting because they involve coevolved populations in which the interacting species have highly specialized traits involved in the mutualisms. Classic examples of obligate pollination are the yucca–moth mutualism and the fig–fig wasp mutualism. But not all mutualisms are obligate and not all obligate interactions are mutualisms. Similarly, not all symbioses are mutualistic and not all mutualisms are symbiotic. Furthermore, mutualisms are not the only association between pairs of species (Currie, 2001; Stachowicz & Whitlatch, 2005; Ossler et al., 2015). The degree of dependency, symmetry, tightness, specificity and specialization vary within and across mutualisms.

2.2. Challenges in understanding the origin and persistence in mutualisms

Mutualisms have long fascinated scientists because of two important questions: how can species that have significantly different evolutionary history and life history traits develop a mutualistic relationship, and how do mutualisms persist through time? The first question is probably the hardest to answer because it relies mostly on speculation based on fossils and phylogenetic evidences. Popular hypotheses are that mutualisms emerged from antagonisms (Thompson, 1982) or from opportunities and chance (Janzen, 1980) but the origin of most mutualisms remain unknown today. Second, the stability of mutualistic interactions has been questioned for a long time. Theoretical approaches utilizing Prisoner's Dilemma game theory to understand mutualisms had, in the past, pointed out their supposed instability due to potential evolution of cheaters (individuals or whole species involved in mutualism), which evolve strategies to increase their fitness by reducing benefits provided to the mutualists, and the emergence of conflict of interests between interacting species (Hamilton, 1964; Trivers, 1971;

May, 1976; Axelrod & Hamilton, 1981; Soberon & Martinez del Rio,1985). The concept that individuals and species tend to maximize their fitness at the cost of the mutualism favors evolution towards a parasitic habit. Empirical observations have confirmed that shifts from mutualisms towards parasitism exists (Thompson & Cunningham, 2002; Sachs & Wilcox, 2005; Sachs & Simms 2006). Furthermore, mutualisms are often subject to exploitation by parasites that were not engaged in the mutualism previously, with differential costs for the mutualism that are potentially destabilizing the interaction. (Bronstein, 2001b; Yu, 2001). However, some mutualisms are known to be very ancient and seem to have been stable over long evolutionary periods (Remy et al., 1994; Machado et al., 2001; Mueller et al., 2001). Several theoretical and empirical studies offer potential ways to account for the stability of mutualists over cheaters and exploiters and their evolutionary trajectories, but the topic is still under debate (Pellmyr & Huth, 1994; Ferriere et al., 2001;West et al., 2002; Kiers et al., 2003; Wilson, Morris & Bronstein, 2003; Holland, DeAngelis & Schultz, 2004; Johnstone & Bshary, 2008; Frederickson, 2013; Heath & Stinchcombe, 2014; Jones et al., 2015).

2.3. Mutualisms in a changing world

Today, the scientific community agrees that an exceptional phenomenon is occuring: a rapid, human-induced, global environmental change which affects wildlife in ways that is not yet entirely understood (Houghton & Woodwell,1989; Vitousek, 1994; Parmesan & Yohe, 2003; Root et al., 2003; Houghton, 2009). Species interactions are expected to be affected by it, and mutualisms are no exception. In fact, species involved in mutualisms are expected to be more affected by the global change than species involved in other types of interactions (Bronstein et al., 2004: Tylianakis et al., 2008; Dunn et al., 2009; Yang & Rudolf 2010). One major discovery

regarding the effects of climate change effect on mutualisms was the differential phenological response to increasing temperature in plant–pollinator interactions. Due to temperature change in the spring, many plants tend to produce flowers earlier (Fitter & Fitter, 2002) while pollinators often respond differently to this change, leading to phenological mismatches between both partners (Walther et al., 2002; Doi, Gordo & Katano, 2008). These phenological mismatches affect not only pollinators, but can alter other mutualisms as well (reviewed in Yang & Rudolf, 2010). Shifts in phenology are not the only concerns, alteration of the environment can affect species interactions in various ways depending on the interaction characteristics. The results can include: mutualism breakdown leading to co-extinction, shifts from mutualism to antagonism, evolutionary switches to novel partners and mutualism abandonment (Bascompte & Stouffer, 2009; Dunn et al., 2009; Kiers et al., 2011).

The current situation is alarming: biodiversity is declining at an unpreceded rate and ecosystem services are vanishing (Worm et al., 2006; Potts et al., 2010; Butchart et al., 2010). Evidence suggests that we are at the beginning of the sixth great extinction, with consequences already being seen (Barnosky et al., 2011; Ceballos et al., 2017). Understanding the biology behind mutualisms, their contribution to biodiversity, their implication in ecosystem and agriculture functioning, and their responses to anthropogenic changes is a challenging task required for successful conservation and potential restoration.

3. Scientific aims, biological model and structure of this dissertation

3.1. Motivation and goals

The major motivation behind the work presented in this dissertation is my fascination with mutualistic interactions and interest in studying their complexity. Mutualisms are

captivating in the way that they represent unexpected positive associations between quite different life forms, providing examples that not all life on earth are solely in a fight against others for their own survival. The idea that only struggle for life (or the famous expression “the law of the jungle”) represent the concepts of competition and natural selection, which are existing phenomenon in nature and important drivers of evolution. I find, however, this expression misleading and obscuring the fact that, in nature, cooperation between species is also ubiquitous. Positive associations concern may be all living beings, and the fact that we are constantly surrounded by both visible and hidden mutualisms (i.e., below ground, within other organisms or microscopic) triggered my curiosity. Along with their underrated importance in the history of life and their crucial current role in ecosystem functioning, they offer diverse, fascinating and instructive biological models to study.

Mutualisms are however challenging to understand. As mentioned in section 2.2, it is hard to explain their origin and persistence over time. Furthermore, the contextual nature of the interactions and the variation of the biotic and abiotic environment across geography raises some fundamental questions: what are the costs and benefits involved in mutualisms? How do these vary spatially within and across populations? What in the environment (i.e., the context) influences the net effect of mutualistic interactions? How does the community of species interacting with mutualists affect them and how do mutualisms impact them in return? How are obligately interacting species structured in geographical space? How did past climatic and geologic events affect and shape the contemporary distribution of interacting species? Did they affect the evolutionary histories of the hosts, symbionts and parasites differentially? What are the consequences on their contemporary genetic structure in the landscape and what will be the consequences on their evolution? How is anthropogenic global environmental change impacting

the current structure of obligate species interactions? Can we predict the direction of change in species interaction given future environmental scenarios? In an era where environmental changes are extremely rapid and biodiversity is collapsing, it is critical to understand mutualist's ecological and evolutionary trajectories, and the consequences on biodiversity, ecosystems and the future of mankind. My goals are to understand how species interactions, in particular mutualisms proceed in a changing environment. I am interested in investigating how historical and contemporary variation in the environment shape the interactions and structure within species communities.

3.2. The fig–fig wasp mutualisms: a textbook case of nursery pollination mutualism

The biological system studied for this dissertation is a case of obligate nursery pollination mutualism (the pollinator offspring feed on flowers, fruits or a subset of seeds): the fig–fig wasp mutualism (Figure 2). Fig trees (*Ficus*, Moraceae) and their associated pollinating fig wasps (family Agaonidae; superfamily Chalcidoidea) represent one of the best-known plant–insect obligate mutualisms, and their relationships are well described (Janzen, 1979). *Ficus* is the largest genus within the Moraceae with more than 750 species (Berg 1989; Cook & Rasplus 2003; Jousselin et al., 2003; Berg & Corner, 2005). Fig trees produce an urn-shaped, nearly closed inflorescence (the fig, which is a multiple fruit called syconium, with a fleshy outer receptable, referred as the wall) which release host-specific volatile chemical cues when receptive to pollinators (Barker, 1985; Chen et al., 2009). Pollen-bearing female fig wasps are attracted by the volatiles and enter the syconium through a small, bract-lined opening (the ostiole). Inside, they disperse pollen on small female flowers and lay eggs into a subset of these and later die inside the fig. Those flowers receiving an egg will be galled by the wasp larva and

will produce the next generation of pollinators, whereas the remaining pollinated flowers will produce single seeded fruits (akenes or nutlets). When the syconium matures, male and female pollinating wasps emerge from their galls and mate within the syconium. The females collect pollen and exit the syconium via a hole chewed in the syconium wall by the male wasps. After the females exits the syconium, the latter increases in moisture and sugar content, attracting frugivores to disperse the seeds. Seed production and dispersal contribute to the female fitness of the fig plant. The production of pollinators benefits both symbionts; male fig fitness is positively affected via the dispersal of pollen. However, the very specialized pollinating fig wasps are short-lived (few days) and have no dormant season, and without a constant supply of syconia to develop in, they would go extinct. For this reason, fig trees require a within-population, year-round production of syconia that prevent pollinating fig wasp from local extinction (Janzen, 1979).

Fig trees and fig wasps are obligate pollination mutualisms which appear to be highly specific. Each *Ficus* species is usually pollinated by only one species of aganoid wasp only. Phylogenetic studies have revealed that *Ficus* and fig wasp species codiverged 60 to 90 Myr ago (Machado et al., 2001; Rønsted et al., 2005). Fig–fig wasp mutualisms have therefore been studied extensively as models of coevolved systems (Herre, 1989; Anstett et al., 1997; Weiblen & Bush, 2002; Machado et al., 2005). However, the one–to–one rule (one host species associated with one pollinator species) is not without exception. Evidence shows host switching and cases where multiple pollinators are associated with one fig tree species or pollinators visit multiple hosts (Weiblen & Bush, 2002; Machado et al., 2005; McLeish & van Noort 2012). Recent findings revealed the existence of up to nine species of pollinating wasps on one fig host (Yu et

al., 2019). The diversity of pollinating fig wasps' ecology and evolutionary biology is large among species (Kjellberg et al., 2005).

Each fig–pollinating fig wasp system is ubiquitously associated with non-pollinating fig wasps that are exploiting the mutualism (Compton & Hawkins, 1992; Bouček, 1993; Borges, 2015). All non-pollinating fig wasps oviposit outside the fig and insert eggs into flowers, the fig wall, other developing wasps (= parasitoid), or the galls induced by other wasps killing their larvae (= cleptoparasites) (West & Herre, 1994; Weiblen, 2002; Elias et al., 2012; Borges, 2015). Thus, they do not provide the service of pollination to the fig. non-pollinating fig wasps are competing with pollinators for floral resource or are parasites of the pollinator or another non-pollinating fig wasp. Their negative impact on the figs and their mutualist pollinator wasps has been demonstrated in several studies (West & Herre, 1996; Kerdelhué & Rasplus, 1996; Cardona et al., 2013; Conchou et al., 2014; Castro et al., 2015). Similar to pollinating species, non-pollinating fig wasps can be specialized host specific organisms (Jousselin et al., 2003; Jousselin et al., 2008) but host switching and multiple host per non-pollinating fig wasp species are also common (Marussich & Machado, 2007).

Figs are thus associated with many specialized species which can be beneficial or antagonistic. Furthermore, the unusual year-round fruiting characteristic of *Ficus* species provides an important source of food for diverse frugivores. Because of these characteristics and the large community of species that rely on figs, *Ficus* species are often important ecosystem components and are recognized as keystone species in the tropics. (Terborgh, 1986; Lambert and Marshall, 1991; Shanahan et al., 2001; Serio-Silva et al., 2002; Harrison, 2005; Kissling et al., 2007)

3.3. *Ficus petiolaris* and its associated obligated insect community in Mexico: the biological model for this dissertation

Ficus petiolaris Kunth is one of the three endemic *Ficus* species of Mexico (Figure 3). First collected in Sinaloa by Bonpland during a five-year expedition, the type specimen was first described by Kunth in 1817 (Stanley, 1917). The reproductive trees range from only a few meters high to magnificent trees as tall as 30 meters (Ibarra-Manríquez et al., 2011; Figure 3). The species is readily recognizable by its yellow bark, the source of its local common name in Spanish "Amate Amarillo". The trees grow specifically on rocky subtrates such as individual rocks, cliffs, river sides or canyon walls. As a result, *F. petiolaris* populations are often dense and patchy, following the pattern of the rocky subtrate. The species has distinctive petiolate cordate -orbicular leaves with tufts of white hair along the leaf veins. The syconia grow in pairs, with a green to red/purple color and long peduncles. Morphological characters are highly variable within the species, to the point where it was first identified as four distinct species *F. brandege*i, *F. jaliscana*, *F. petiolaris* and *F. palmeri*. However, a recent study suggests that they all belong to one species only, with a wide range, taking the name of the first of these subspecies to be described: *Ficus petiolaris*.

Ficus petiolaris is one of three *Ficus* endemic species to Mexico, with a relatively large range which extends from the northwest of Mexico including Baja California and Sonora, to the southern coast of Oaxaca (Serrato et al., 2004; Piedra-Malagón et al., 2011). The hymenopteran fauna associated with *F. petiolaris* has been identified to genera (Duthie et al., 2015; 2016; Piedra-Malagón et al., 2018). The pollinator belongs to the genus *Pegoscapus*, Cameron 1906, a group of chalcid wasps which pollinate fig trees from the subgenus *Urostigma*. Antagonists to the mutualism are four species of *Idarnes* Walker (family Agaonidae, two from the species-group *carme* and two from the species-group *flavicolis*), two species of *Heterandrium* Mayr

(family Pteromalidae), one species of *Ficicola* Heydon (family Pteromalidae), and its parasitoid *Physothorax* (family Torymidae), and one rarely observed species of *Sycophila* Walker. *Ficus petiolaris* is also associated with other antagonists: a lepidopteran larva which causes tremendous damage to figs (Piatscheck et al., 2018), and nematodes that parasitize the pollinating wasp, as well as the non-pollinating fig wasps (Van Goor et al., 2018). A representation of the *F. petiolaris*–pollinators–antagonists system is shown in Figure 4.

3.4. Organization of this dissertation

My motivation is to gain understanding of the ecological and evolutionary dynamic of an obligate species community, with the goals of inferring historical processes that shaped their current distribution, and exploring ecological aspects of a variable environment on community structure. By gaining insights on the effects of past and contemporary factors on the species' dynamics, I expect to forecast the potential changes of this community into the future. The *F. petiolaris*–pollinating wasp–non-pollinating fig wasps–frugivore system provides an excellent biological model to investigate the scientific questions I would like to investigate.

In **Chapter 2** I investigate the effects of deep- and shallow-time geological and climatic factors that have potentially affected the evolutionary history of *F. petiolaris* and shaped its current genetic structure by studying its phylogeography across its range in Mexico. Using species distribution modeling, I hypothesize *a posteriori* that Pleistocene climate oscillation had critical effects over potential vicariance breaks on the history of *F. petiolaris* in Mexico, and consequently their current genetic landscape. With DNA samples from 203 individuals in 19 populations of *F. petiolaris*, I used genetic clustering, population genetics and phylogenetics analyses to address these hypotheses. I found that the last glacial maximum induced range shift

southward resulting in a large refugial population along the southern coast of Mexico and a small refugium in Baja California, followed by northward colonization. This work laid the groundwork for a co-phylogeographic study of *F. petiolaris* and its associated obligate insects.

In **Chapter 3**, I study the effects of environmental variations on the reproductive dynamics of the pollinating fig wasps and non-pollinating fig wasps. I develop a set of hypotheses in which biotic and abiotic factors could profit or disadvantage fig wasp species. The ultimate goal is to identify what environmental context favors pollinators over non-pollinating fig wasps. With extensive field work over four field seasons at nine sites in Baja California, we measured a set of eight biotic and abiotic tree-height variables and described the geographic and temporal variation of the fig wasp community associated with the environment. Results indicate a highly variable system in which fig wasp species are associated in different ways. The conclusions provide insights on environmental consequences to pollinator–non-pollinating fig wasp dynamics. Conditions for mutualism maintenance are discussed.

Chapter 4 presents a study on the effects of *Omiodes stigmosalis*, a specialized lepidopteran larva that feeds on developing syconia and causes dramatic damages to fig tree crops. After identification of the lepidopteran fig frugivore and characterization of the spatial aggregation of the damage on fig trees, I hypothesized that phenology, quantity and spatial distribution of *F. petiolaris* would be associated with variation in lepidopteran damage. I found that a large crop with highly synchronous reproduction favors the lepidopteran. The reproductive strategies of *F. petiolaris* and their consequence on seed and wasp production are discussed.

Finally, in **Chapter 5** I use the knowledge gained in Chapter 2 to predict fig wasp community dynamics into the future. Using species distribution modeling with a future averaged climate scenario for moderate and pessimistic greenhouse gas concentration scenarios and

ecological modeling, I project the future habitat suitability of *F. petiolaris* and expected composition of pollinators relative to non-pollinating fig wasps. The model projects a shift of *F. petiolaris*' distribution northward and predicts an environmental condition that will globally favor the non-pollinating fig wasps over the pollinator, and it allows visualization of species community composition over geographic space in Mexico. This technique of species interaction dynamics forecasting allows identification of regions where conditions are predicted to be the worst for the mutualism. Here we show that statistical modeling allows prediction of the effect of anthropogenic global climate change on species interactions into the future.

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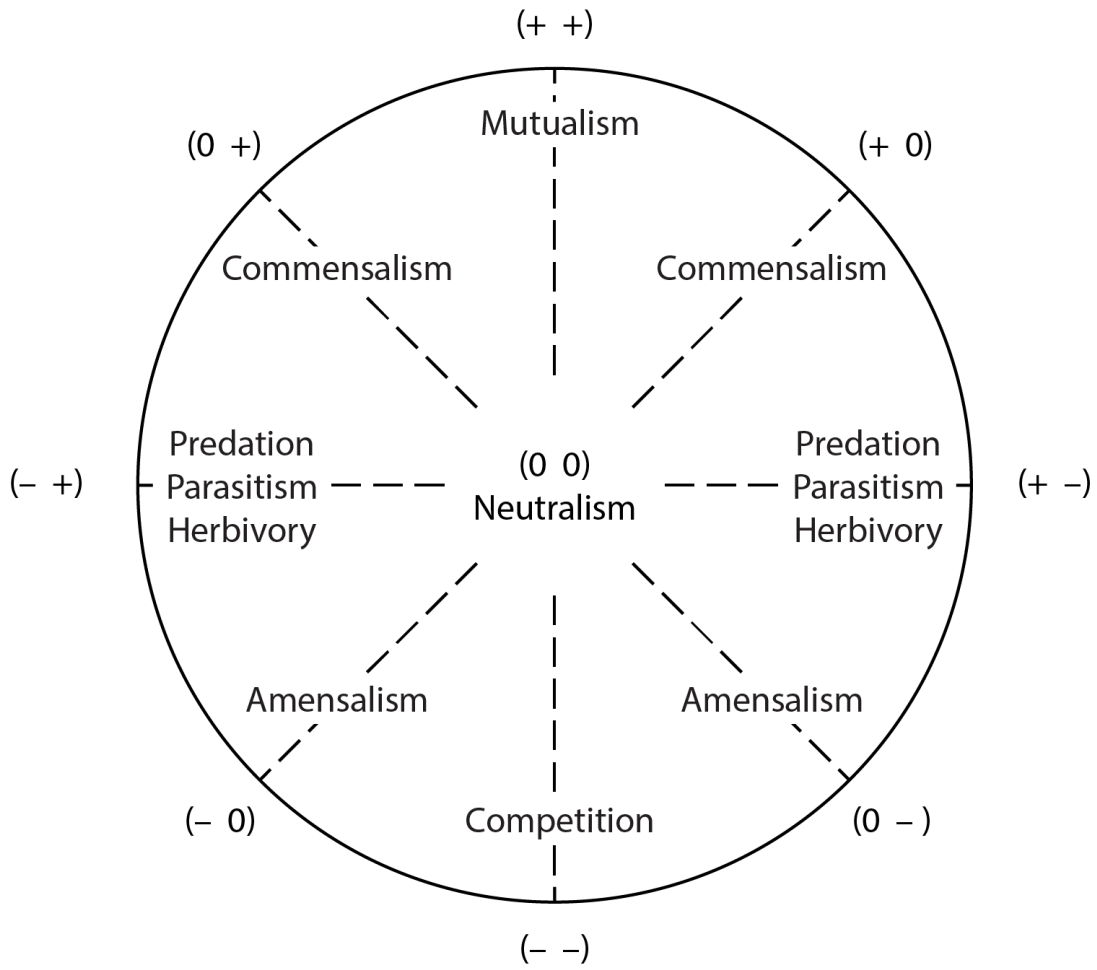

Figure 1. The coaction compass, representing all possible interaction between pairs of species. Symbols 0, + and − represent net effect of the interaction. This representation shows how interactions grade into each other. Antagonism refers to any interaction type with –. Modified from Bronstein (2015).

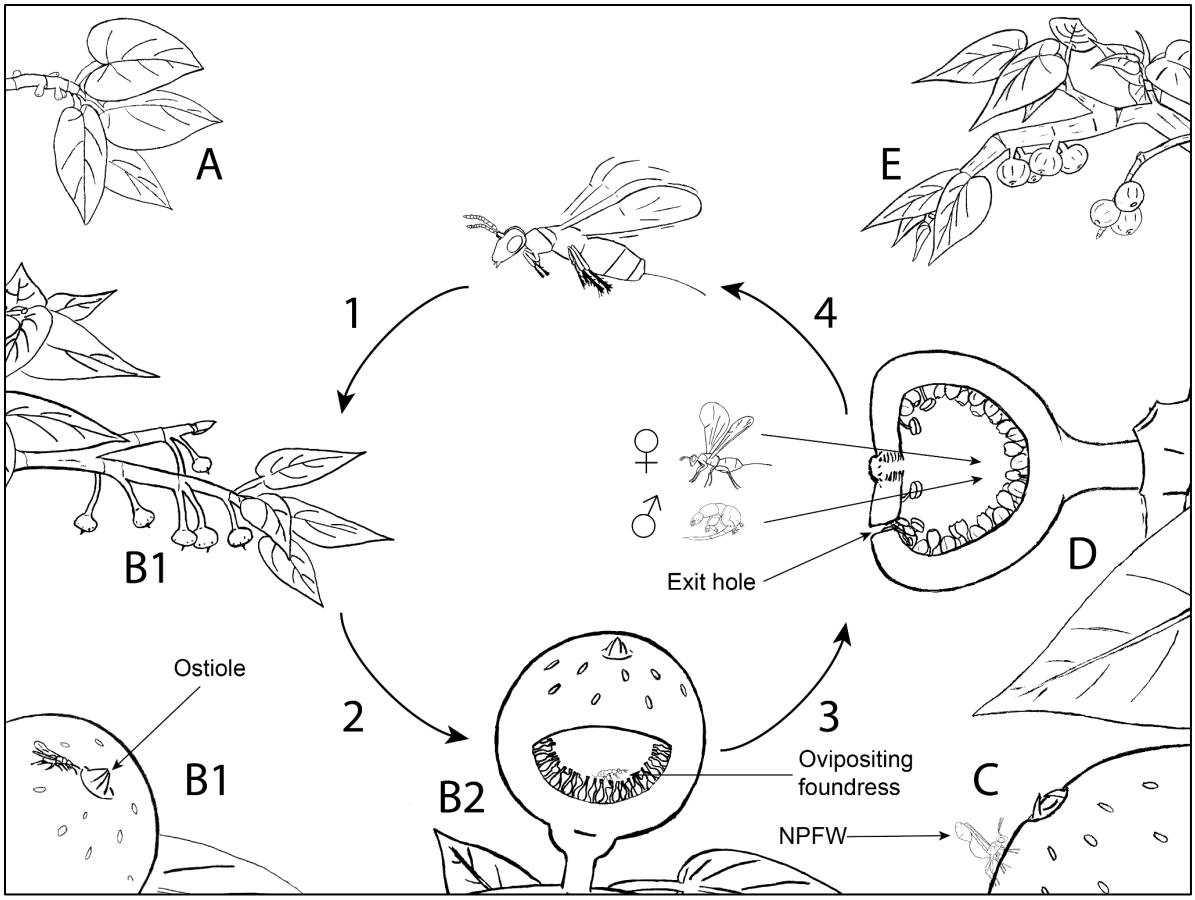

Figure 2. The fig–fig wasp symbiotic mutualism in monoecious fig trees illustrated with the *F. petiolaris* system. Letters describe parts of the reproductive cycle of the fig tree, with references to the syconium phases described by Galil & Eisikowitch (1968a and 1968b). Numbers represent parts of the fig wasp life cycle. A: syconia are pre–receptive (phase A), female flowers are developing and the ostiole is closed; B1: syconia are receptive (phase B), female flowers have well developed styles, the ostiole is open, chemical clues are released that attract fig wasps; 1: a (or several) female pollinator (*Pegoscapus sp.*) carrying pollen flies to the receptive syconia; 2: the pollinating wasp lands on the receptive syconium (B1) and enters it through the ostiole; B2: female flowers are pollinated or infested with wasp eggs, the ostiole closes, the foundress(es) dies; C: the syconia mature and are in an interstate (C phase), fertilized flowers develop into fruits (likely nutlets in *F. petiolaris*, F. Piatscheck, pers. obs.), non–pollinating fig wasps (an *Idarnes* female in the illustration) lay eggs in flowers, galls or the inflorescence receptacle without entering the syconium. Note: non–pollinating fig wasps also lay eggs in B1 or B2 syconia. 3: Fig wasps mature; D: fruits and male flowers are developed (D phase); 4: male and female wasp offspring (pollinators and non–pollinating fig wasps) emerge and mate, *Pegoscapus* females take pollen from male flowers, *Pegoscapus* males dig an exit hole in the flesh of the syconium allowing wasp to escape to fly to other syconia; E: syconia are ripe (E phase) and produce carbohydrates, fall off the tree or are dispersed by vertebrates. Note: an F phase has been described that represents the role of syconia after they fall from trees, but is not represented in this illustration (Palmeiri & Pereira, 2018).

Figure 3. *Ficus petiolaris* and its pollinating wasp. A) *Ficus petiolaris* large reproductive tree at site "96"; B) asynchronous flowering with pairs of syconia from reproductive phase A, B and C in Fig. 2; C) electron microscopy of female *Pegoscapus* sp. with pollen artificially colored in yellow; D) electron microscopy of male *Pegoscapus* sp. Photography of *F. petiolaris* by J. D. Nason, *Pegoscapus* sp. electron microscopy from Piedra-Malagón et al., 2018.

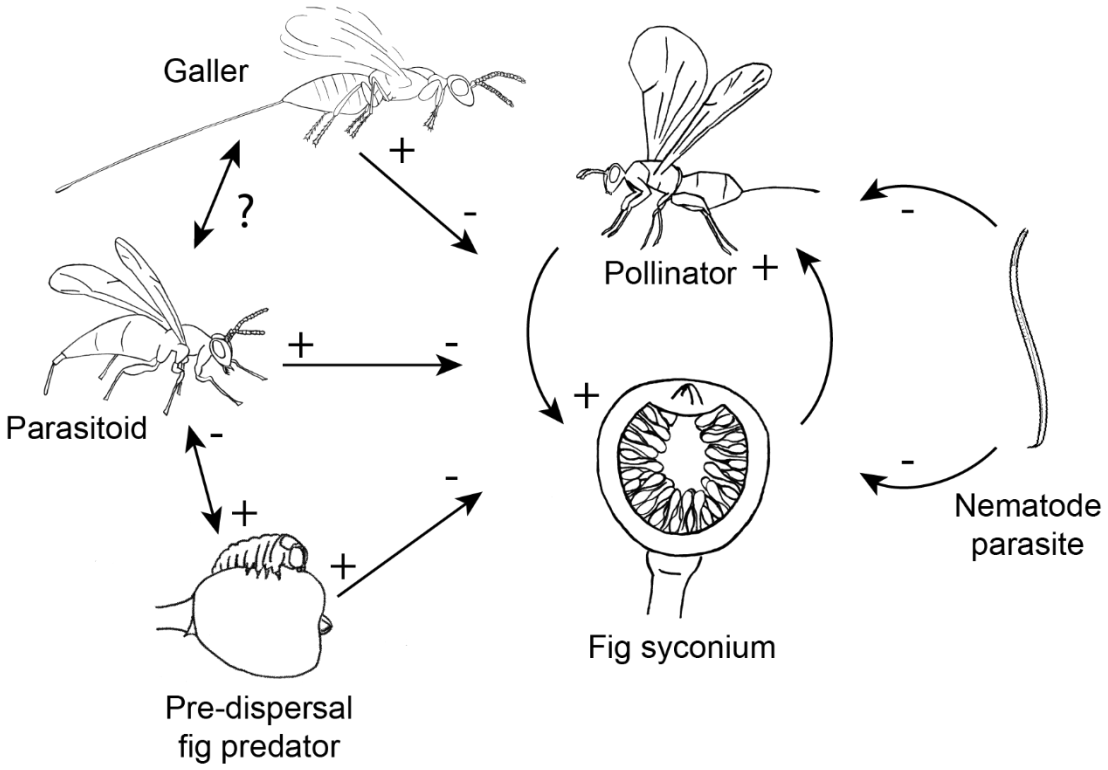

Figure 4. Schematic representation of interactions in the *F. petiolaris* obligate mutualism–antagonism system. Pollinator = *Pegoscapus* sp. female wasp; Galler = *Idarnes carme* sp. 1; Parasitoid = *Heterandrium* sp. 1, Pre-dispersal fig predator = lepidopteran larva. Signs represent the assumed net effect of the interaction on each species. Other non–pollinating fig wasps and hypothesized parasitoids of non–pollinating fig wasps are not represented for simplicity.

CHAPTER 2. PHYLOGEOGRAPHY OF FICUS PETIOLARIS, A WIDESPREAD MEXICAN ENDEMIC FIG TREE

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Abstract

Historical vicariance has had major effects on the geographical genetic structure of many organisms. However, at the interface of temperate and subtropical regions, the distribution and phylogeography of frost-sensitive plants may have been more responsive to late-Pleistocene climatic fluctuations than that of vertebrates. Here we were interested in environmental processes at deep and shallow time scales that have affected the biogeography and population genetics of *Ficus petiolaris,* an endemic fig tree of Mexico. We tested existing hypotheses of deeper time sources of vicariance spanning the distribution range of *F. petiolaris*, and inferred habitat suitability during the last glacial maximum (LGM; ca. 22,000 years ago) to generate and test *a posteriori* hypotheses of shallower-time changes to the species' distribution. We sampled 19 populations across the range of *F. petiolaris* and used restriction site-associated DNA sequencing to generate 727 polymorphic ingle nucleotide polymorphism loci, which were used to test phylogeographic hypotheses. *F. petiolaris* in Baja California represents a distinct phylogroup

that has experienced a genetic bottleneck, but did not exhibit the expected signal of northward range expansion from a southern refugium. Populations in interior northwestern Mexico cluster genetically with central Mexico suggesting colonization from the south, while coastal northwestern Mexico populations show clear admixture between Baja California and central Mexico phylogroups. The expected vicariance between populations in central Mexico and southern Mexico (Oaxaca) was strongly supported by genetic clustering using PCA but not using model-based clustering that includes a spatial component (*conStruct* in R). We conclude that like other subtropical plants in Mexico, the northern limits of the *F. petiolaris* range shifted southward during the LGM, with subsequent range expansion resulting in a contemporary contact zone in coastal northwestern Mexico between previously isolated Baja California and mainland Mexico phylogroups. Central and southern Mexican populations maintain high levels of genetic diversity consistent with stable effective population sizes through time.

1. Introduction

Spatial and temporal environmental fluctuations are expected to affect species' distributions and migration rates (Pease et al., 1989; Hewitt, 1996; Soltis et al., 1997; Riddle et al., 2000). Due to the heritability of DNA, demographic processes associated with changes in biogeographic patterns shape the frequencies of alleles of organisms in space and time. The resulting distribution of alleles across loci and individuals provides essential information that can be used to determine population structure (Pritchard et al., 2000) and infer past demographic processes (Salmona et al., 2017; Beichman et al., 2018). In a geographical context, this results in a genetic landscape that can reveal the mechanisms responsible for the observed biogeographic pattern. Phylogeography aims to link geological and historical factors with the spatial

distribution of gene lineages within and among closely related species (Avise, 2000). Phylogeographic methods include phylogenetic and population genetic analyses applied in a spatial context, and have been increasingly used by scientists to explore species' demographic histories and to highlight ongoing ecological and evolutionary processes (Beheregaray, 2008). Thus, by investigating a species' genetic landscape, we can acquire insights into underlying historical processes and current patterns of gene flow, and likewise the demographic processes that led to contemporary biogeographic patterns.

Vicariance and dispersal are often invoked as having strong influences on genetic relationships among natural populations (Ronquist, 1997; Futuyama, 1998). Within the range of a species, discrete events such as separation by physiographic barriers can limit dispersal between localities and can result in gradual genetic differentiation between populations due to genetic drift. Such barriers, which result in isolated populations or vicariance, have important evolutionary consequences as they lead to population differentiation and, potentially, speciation events. Alternatively, gene flow promotes homogenization of genetic variation between localities which can reduce population differentiation and, potentially, local adaptation (Slatkin, 1985). Historical contraction and expansion in species ranges can also have consequences for contemporary spatial demography and genetic variation (Hewitt, 1996). These range-shifting events can erase the genetic signatures of more ancient sources of vicariance. Careful interpretation of phylogeographic patterns with respect to *a priori* evidence from plate tectonics, taxonomy, and hind-cast species niche modeling is essential to gain robust insight into species historical distributions and distinguish between ancient vicariance or more recent dispersal processes. The difficulty remains that many other factors variation (e.g., selection, genetic drift and/or stochastic gene lineage sorting) affect the evolution of species and consequently their

spatial genetic. However, major demographic processes should leave a genetic signature in a species' genetic landscape, and the use of rigorous hypothesis generation and testing is necessary to correctly interpret phylogeographical patterns (Knowles, 2004).

The United Mexican States (referred as Mexico in the following) is a country with complex geological and volcanic histories contributing to a complicated physiography (Morán,1986; Lugo-Hubp, 1990; Ferrusquia-Villafranca, 1993; Macías, 2005). Major physiographic features include several major mountain ranges, a large central plateau, peninsulas, isthmuses and many islands (Ordóñez, 1941). Mexico is also highly diverse biologically (Toledo & Ordóñez, 1998; Mittermeier & Goettsch, 1992; Myers et al., 2000; Luna Plascencia et al., 2011), and together with 12 other "megadiverse" countries accounts for 70% of the world's biodiversity (Llorente-Bousquets & Ocegueda, 2008). Contributing to the origins and maintenance of this biodiversity, Mexico is located at the transition between the Neotropical and Neararctic realms (Wallace, 1976), encompasses highly variable climates (García, 1988), and supports many vegetation types, ranging from arid desert vegetation to evergreen tropical rainforest (Rzedowski, 1986). Because of these abiotic and biotic features, as well as climatic fluctuations during the Pleiestocene, the biogeographies of contemporary Mexican species can be complex, especially for those with broad geographic distributions.

Within Mexico, fig trees (genus: *Ficus*; family: Moraceae) are widely distributed, and are especially diverse in tropical forests of the southern part of the country, but are also commonly found in grasslands and xeric shrublands (Serrato et al., 2004). *Ficus* is the largest genus in the family Moraceae with 750+ species distributed worldwide (Berg, 1989; Cook & Rasplus, 2003; Jousselin et al., 2003; Berg & Corner, 2005). Fig trees can be woody bushes to canopy-emergent trees, all having a unique reproductive biology involving interactions with pollinating fig wasps

(family Agaonidae; superfamily Chalcidoidea), with which they have been coevolving for ca. 80 million years. These specialized wasps are the only pollinators of fig flowers, and the fig-wasp interaction is usually highly species-specific. All figs produce unique inflorescences called syconia, urn-shaped inflorescence with small flowers within a hollow receptacle accessed by an opening called the ostiole. Pollinating fig wasps enter the inflorescence, pollinate female flowers, and lay eggs of their offspring into a subset of flowers. Seeds and wasps develop within syconia, which mature to become "fruit". The interaction between fig and wasp is mutualistic, with a fig species relying on its associated wasp species for pollination services, and the wasp relying on the fig's flowers for the development of its young. Because adult pollinating fig wasps live only a 2-3 days and rely on syconia for reproduction, fig populations must produce syconia year-round to avoid the local extinction of its pollinator (Janzen, 1979). This aseasonal production of fig fruit constitutes an important nutritive resource for a diversity of vertebrate herbivores, especially during seasons when the fruiting activity of other plants is low (Terborgh, 1986; Lambert & Marshall, 1991; Shanahan et al., 2001; Serio-Silva et al., 2002; Harrison, 2005; Kissling et al., 2007). Because of these features, fig trees are recognized as important contributors to ecosystem function (i.e., a keystone species).

Three of the 22 *Ficus* species in Mexico are endemic to the country (Ibarra-Manríquez et al., 2011). One of these endemic species, *F. petiolaris* (subgenus: *Urostigma*, section Americana), is particularly widespread, with a range extending from the states of Baja California and Sonora in the northwest to Oaxaca in the southeast. It is the northernmost *Ficus* in the new World and the only truly desert adapted Mexican fig, occurring throughout Sonoran Desert habitats in Baja California and Sonora (Serrato et al., 2004; Piedra-Malagón et al., 2011). The species exhibits substantial morphological variation among individual trees across its geographic

range, which in the past led taxonomists to classify *F. petiolaris* morphs into four species (*F. brandegeei, F. jaliscana F. petiolaris* and *F. palmeri*). However, based on the analysis of several morphological characters, they were later reclassified as subspecies within *F. petiolaris* (Felger et al., 2001; Piedra-Malagón et al., 2011). Nonetheless, the phylogeography of *F. petiolaris* remains virtually unknown.

Several major physiographic features are potential barriers to gene flow across the range of *F. petiolaris* (Figure 1): the Sierra Madré del Sur and Trans–Mexican Volcanic Belt in the south, and the Sierra Madré Occidental and Baja California peninsula to the northwest. The 1000 km long Trans–Mexican Volcanic Belt is the highest physical barrier in Mexico, is about and formed between 11Myr and 3Myr (Ferrari et al., 2012). Volcanic activity is ongoing today. The Trans–Mexican Volcanic Belt has greatly contributed to the biogeographic histories of many taxa by acting as a barrier to gene flow and subsequence population divergence. (Darda, 1994; Sullivan et al., 2000; Mateos, 2005; Mulcahy et al., 2006; Bryson et al., 2011a; 2011b; Parra-Olea et al., 2012; Ruiz-Sanchez & Spetch., 2013; 2014;). The climatic and volcanic history of the Trans–Mexican Volcanic Belt is complex, however, and the history of highland species can be challenging to reconstruct (Mastretta-Yanes et al., 2015). Similar observations were made for taxa distributed along the Sierra Madre Occidental (Riddle et al., 2000; Jaeger et al., 2005; Schönhuth et al., 2011, Bryson et al., 2011a), a large mountain range formed 38-20 Myr ago (Ferrari et al., 2002). South of the Trans–Mexican Volcanic Belt, the Sierra Madre del Sur formed 48-23 Myr ago (Ferrari et al., 2014), and represents another complex mountain range within the distribution of *F. petiolaris*. This range extends northwest to Sonora and to Baja California (but does not connect across the northern limits of the Sea of Cortez), which also have a rich geological history. On the Baja California peninsula, *F. petiolaris* was long identified as *F.*

palmerii but has, since, been recognized as a geographically isolated *F. petiolaris* sub-species (Piedra-Malagón et al., 2011). Baja California was originally part of the Californian plate before separation by the Sea of Cortez. The trans-peninsular seaways subsequently crossed Baja California, which also experienced substantial volcanic activity, leading biologists to investigate several hypothetical sources of vicariance (Figure 1): the separation of the Baja California peninsula from continental Mexico following the formation of the Sea of Cortez (ca. 5 Myr ago), an oceanic inundation of the Isthmus of La Paz (ca. 3 Myr ago), and a more recent mid-peninsular seaway (ca. 1 Myr ago). These sources of vicariance have been confirmed by several animal species (Upton & Murphy, 1997; Hurtado et al., 2004; Riddle et al., 2000; Lindell et al., 2006; Riddle & Hafner, 2006; Crews & Hedin, 2006; González-Rubio et al., 2016), which in some cases have been attributed to climatic conditions (Graham et al., 2013). Two other physiographic barriers could also limit dispersal in *F. petiolaris*: the Sierra Barabampo-Rio Fuerte in Sinaloa as proposed in Riddle & Hafner (2006) and interpreted as climatic differences by Edwards et al. (2016), and a vicariance break observed in Baja California located in the region of Loreto as mentioned in Lindell et al., (2005). Dates for these latter two sources of vicariance have not been hypothesized.

With the exception of *Nolina parviflora* and the Trans–Mexican Volcanic Belt, the vicariance breaks in the studies cited above concern animal species. Unlike animals and their potential for behaviorial acclimatization, individual plants cannot avoid exposure to adverse weather caused by changing climate. For example, animals have means for thermoregulation which plants to not, especially frost sensitive plants of tropical or subtropical origin. Phylogeographic studies on plants, notably in Baja California reveal dissimilar phylogeographic patterns than observed in animals (Nason et al., 2002; Clark-Tapia & Molina-Freaner, 2003;

Garrick et al., 2009). These studies suggest that past shifts in the distribution of the suitable ecological niches of species due to environmental changes, notably Pleistocene glacial-interglacial cycle contractions and expansions, may have impacted plants more than animals. Glacial fluctuations during the Quaternary are more recent than physiographic factors identified as sources of vicariance (last glacial maximum (LGM) ca. 28-19 Kyr, with cold climatic conditions lasting until ca. 14.5Kyr, Clark et al., 2009). The consequences for plants could be southward range contraction to refugial populations followed by northward post ice age colonization. In Baja California plants, this history of range contraction and expansion has been associated with higher genetic diversity in southern refugial populations and declines in genetic diversity with increasing latitude (Nason et al., 2002; Garrick et al., 2009). These changes in distribution may overwrite the genetic signatures of earlier vicariance, if so, genetic variation across the landscape should exhibit signature of more recent demographic processes.

Here we investigate historical processes influencing the phylogeography of *F. petiolaris*. We assess *a priori* hypotheses of vicariance resulting from current physiographic and past geological features (Figure 1). However, because changes in distribution should also be considered, we compared the current distribution of *F. petiolaris* (Figure 1 and Supplemental Figure 2) with predicted habitat suitability during the LGM using three different scenarios and an ensemble averaged scenario (Figure 2; methods are described in section 2.3, results detailed in section 3.1). From this comparison we formulate additional, *a posteriori* hypotheses (Figure 3): (1) Southward range contraction on the Baja California peninsula during the LGM followed by northward range expansion from refugial populations in Baja's Cape Region, possibly associated with a population and genetic bottleneck. (2) Southward range contraction in northwestern continental Mexico during the LGM followed by northward range expansion from extensive,

genetically diverse populations in central Mexico into present day Sinaloa and Sonora. (3) Habitats in southern Mexico, in particular along the coast, remained favorable for *F. petiolaris* from the last glacial maximum to the present, with large population sizes supporting high genetic diversity. We tested vicariance and distributional hypotheses concerning phylogeography and genetic structuring using genomic data obtained from a modified double digest restriction-site associated protocol (ddRADSeq; Peterson et al., 2012) applied to samples from 19 populations of *F. petiolaris* distributed across the species' range in Mexico.

2. Materials and methods

2.1. Study system, populations and sampling

Ficus petiolaris is a monoecious, rock-strangler fig tree, which grows only on rocky substrate such as rock patches, cliffs or canyon walls, forming naturally small but dense patchy populations, which is unusual for fig trees of the tropics in the new world. In the peninsula of Baja California, *F. petiolaris* trees are used by locals for nutritive and medicinal purposes. Its distinctive morphology (yellow bark and year-round leaf production unlike most desert plants) is appreciated and sometimes used to host shrines for religious purpose (F. Piatscheck, pers. obs.). Ecologically, *F. petiolaris* seems to have an important role in the desert of Baja California particularly during the dry seasons because it produces leaves and occasionally syconia which can host and feed desert fauna. During summers, the trees are covered by insects while neighboring plants are usually leafless and do not host many insects (J.D. Nason, pers. obs.). Morphological and phylogenetic studies have placed *F. petiolaris* in the subgenus *Urostigma*, section Americana (Cruaud et al., 2011). Its associated fig wasps have been identified to the genus using Bouček's key to chalcidoid wasps associated with *Ficus* in the New World (1993)

but have not been described yet. As with many other *Ficus* species, we lack knowledge about *F. petiolaris*' biology, in particular its functioning in arid conditions, its interactions with local fauna and its evolutionary history.

Ficus petiolaris has recently gained some attention in the scientific literature. *F. petiolaris* exhibits a singular year-round within-tree asynchronous flowering (exhibited in some but not all individual trees), and its reproductive phenology has been studied to elucidate its interaction with its symbiotic pollinating fig wasp, *Pegoscapus* sp. Within-tree asynchrony allows receptive and wasp releasing syconia to overlap in time, assuring reproductivion with the pollinator entering and pollinating syconia of their natal tree, but seemingly at the cost of selfing (Smith & Bronstein, 1996; Gates & Nason, 2012). However, molecular analyses revealed little inbreeding in *F. petiolaris* suggesting an absence of selfing in this biological system (Gates & Nason, 2012). Furthermore, pollinating wasps have excellent dispersal capabilities, utilizing wind to reach fig trees located far away from their natal tree (Nason et al., 1998), and personal observations suggests that *F. petiolaris*' pollinating wasps avoid entering syconia on their natal trees. Both mutualists have natural enemies: specific non-pollinating fig wasps which have been used to develop model of coexistence in fig wasp communities (Duthie et al., 2014; 2015; 2016), parasitic nematodes which impact both pollinating and non-pollinating fig wasps (Van Goor et al., 2018) and a lepidopteran pre-dispersal seed predator whose attack rate is correlated with phenological variables of *F. petiolaris* (Piatscheck et al., 2018). Syconium and fig wasp morphological and anatomical characteristics were recently documented from two trees in the state of Michoacán (Piedra-Malagón et al., 2018). Besides these recent advances, many gaps remain in our understanding of this mutualistic system, in particular the past and recent

evolutionary histories of *F. petiolaris* and its associated specific symbionts which are virtually unknown.

Populations of *F. petiolaris* are located in Baja California Norte, Baja California Sur, Sonora, Jalisco, Zacatecas and Oaxaca (Figure. 4). Populations from Baja California were sampled during 2012, 2013 and 2014 as part of field work associated with other studies. Mainland Mexico's populations were prospected and sampled as part of field work in 2017 in order to improve sampling across *F. petiolaris'* range. The result was greater representation from its range in Baja California with 12 populations including three populations from Sonora across the sea of Cortez, two populations from "Central" Mexico (Jalisco and Zacatecas) and two from southern Mexico in the state of Oaxaca. Population coordinates and sample sizes are indicated in Table 1.

Leaf samples were collected opportunistically by the Nason lab from 2012-2017. Leaves were gathered, cut into strips, then dried with silica gel and preserved in cold conditions (ice chests) on the day of collection. Silica gel was replaced as needed until leaf tissue was sufficiently dried. The samples were then transported to Iowa State University and stored at -80°C until processing for molecular analyses.

2.2. *Ficus petiolaris* occurrence data

Occurrence data of *F. petiolaris* were obtained from georeferenced samples collected by the Nason lab, and database coordinates from GBIF, iDigBio and iNaturalists extracted using *rgbif*, *spocc* and *rinat* in R (R Core Team, 2019) respectively (Chamberlain et al., 2016; 2018; Barve & Hart, 2014). Database searches were conducted using the taxonomic names: "*Ficus petiolaris*","*Ficus palmeri*","*Ficus brandegeei*" and "*Ficus jaliscana*" and coordinates were

selected when present. Occurrence data were then filtered to remove ambiguous locations (i.e., 0° latitude and 0° longitude, or other unexpected locations), and retained only if within Mexico. Ambiguous samples within Mexico out of the current known range of *F. petiolaris* were also removed: they consisted mostly of samples east of the isthmus of Tehuantepec, of which some were visited and identified as *Ficus aurea.* Common misidentifications of *F. aurea* as *F. petiolaris* in the region was confirmed by Dr. Guillermo Ibarra (Universidad Nacional Autónoma de México). Within the remaining 3,329 occurrence coordinates, duplicates and those not located on dry land were removed. The remaining 2,128 occurrence data were used for further niche modeling (see section 2.5), and to create the range of *F. petiolaris* with the package *conR* using the alpha hull method (Dauby et al., 2017). Administrative boundaries were obtained from Global Administrative Areas (2012) and elevation from SRTM (Farr et al., 2007) and plotted using *raster* (Hijman et al., 2015) and *ggplot2* (Wickham, 2016).

2.3. Pleistocene habitat suitability modeling

Occurrence data were filtered in R (R Core Team, 2019). Even after removal of duplicate location coordinates, the occurrence data still presented a large sample bias, mostly due to populations we visited in which we visited where dense patches of trees were georeferenced within short distances. To remove the sample bias, we projected the 2128 occurrences with their spatial coordinates on a 0.5*0.5-degree resolution grid and randomly sampled one occurrence in each cell. After bias removal, the remaining 140 occurrence coordinates that were left had a uniform representation in space. Because we had presence data only, we randomly generated pseudo-absence data in grid squares two degrees larger than *F. petiolaris*' range over that land surface of Mexico. Because Barbet-Massin et al., (2012) recommended a large amount of

background data, we generated randomly 10,000 pseudo-absence points.

Global averaged climate data were obtained from Worldclim v2.0. We used the 19 bioclimatic variables for both current climatic conditions and for the LGM (Worldclim v1.4). As correlation between bioclimatic variables is not recommended, we eliminated every bioclimatic variable with a pairwise correlation with another higher than 0.7. When choice was needed to be done to retain one or another variable, we arbitrary chose the one we believed more impactful for *F. petiolaris*' biology. Retained bioclimatic variables were: Bio1 (Annual Mean Temperature), Bio2 (Mean Diurnal Range), Bio3 (Isothermality), Bio5 (Max Temperature of Warmest Month), Bio8 (Mean Temperature of Wettest Quarter), Bio9 (Mean Temperature of Driest Quarter), Bio12 (Annual Precipitation) and Bio14 (Precipitation of Driest Month). Past climatic condition data for the late Pleistocene are based on three scenarios, CCSM4, MIROC-ESM and MPI-ESM-P. We also created an averaged "ENSEMBLE" scenario that used the average of each paleo-bioclimatic variable for the three models. Occurrence and current bioclimatic data were used to train several models with the package *sdm* (Naimi & Araujo, 2016). Models used were generalized linear model (GLM; Nelder & Wedderburn, 1972), generalized additive model (GAM; Hastie & Tibshirani, 1990, gradient boosting machine (GBM; Friedman, 2001), support-vector networks (SVM; Cortes & Vapnik, 1995), random decision forests (Random Forest; Breiman, 2001), Multivariate adaptive regression splines (MARS; Friedman, 1991), and maximum entropy models (Maxent; Phillips et al., 2006). Fitted models were evaluated using 30% of the initial dataset to test the trained model for three iterations. Models performance was judged based on several criteria: percentage of occurrences fitted in the model, mean area under the ROC curve (AUC; Fielding & Bell, 1997) and mean true skill statistic values (TSS; Allouche et al., 2006). These statistics indicate better performance of the model at higher values (range: 0-

1). AUC has been criticized to vary depending on the extent of the data but the TSS statistics is usually trusted for model evaluation over that of the Kappa statistic, as Kappa has been shown to be overly dependent on prevalence. We report both statistics in Supplemental Table 1. Because species distribution models may provide very different results, making it difficult to choose which model to use, we followed the recommendation to use the ensemble of the models for suitability projection (Shabani et al., 2016). Thus, we retained all models that were successful and created a model weighted by the TSS scores to project present habitat suitability. Past species' habitat suitability was predicted using the weighted models parameterized with paleo-climatic variables and their averaged ensemble. The projected past suitability scores were compared between present and past paleo-climatic conditions to observe potential past shifts in *F. petiolaris*' range, identify potential refugia and help us in formulating hypotheses.

2.4. Genome sequencing and SNPs calling

We obtained single nucleotide polymorphism (SNP) data from deoxyribonucleic acid (DNA) extracted from *F. petiolaris* for phylogeographic analyses. First, we extracted the DNA from all samples, aiming for DNA yield above 20ng/µl. We used a DNA extraction protocol with cetyltrimethylammonium bromide (CTAB) and chloroform which turned out to be the most efficient to obtain high yield of DNA when compared to other DNA extraction methods. DNA was precipitated in ß-propanol instead of commonly used ethanol, which increased DNA precipitation, but with the downside of increasing precipitation of other chemical compounds too. To increase DNA yield and quality, we incorporated polyvinylpyrrolidone and proteinase K in the protocol We modified the protocol of Peterson et al. (2012) which allows the selection and sequencing of double digested DNA fragments with the restriction enzymes PstI and MspI (dd-

RAD sequencing). The digested products were ligated at each cut sites by the restriction enzymes with Illumina ® adapters. Because DNA quality varied across samples, we performed polymerase chain reactions of double digested DNA fragments of each sample separately and confirmed successful amplification via electrophoresis. The same protocol was applied for eight outgroups from six different *Ficus* species: *F. carica*, *F. sycomorus*, *F. pertusa*, *F. citrifolia*, *F. tonduzii*, *F. insipida*, *F. yoponensis*.

The recently sequenced genome of *F. carica*, revealed a genome size of approximatively 700Mb (for the diploid genome) consisting of 33% GC base pairs (Mori et al., 2017). We used the R package SimRAD to simulate a genome with these parameters and conducted an *in-silico* digestion. Based on the results of this simulation, we selected double-digested fragments within a 300bp and 800bp size range, which would result in approximatively 30,000 DNA fragments per individual assuming complete digestion. Polymerase chain reactions products were pooled at equal concentrations, size-selected with BluePippin ® and DNA libraries were checked with Bioanalyzer ®. DNA libraries were quantified and pulled together for later sequencing at the Iowa State University sequencing facility with an Illumina ® HiSEQ 3000 sequencer. Raw reads were sorted, filtered and assembled using the software ipyrad (Eaton & Overcast, 2016) which allows for discovery of loci with SNPs. We applied a maximum of five low quality base calls with the quality score offset of a minimum of 33, minimum depth for statistical base calling of six, minimum depth for majority-rule base calling of six and a maximum cluster depth within samples of 10,000. Reads were assembled together *de novo*. Loci were further filtered, to remove loci found in less than 50% of the samples, and allowing a maximum of 25% heterozygous sites per locus, maximum of five SNPs per locus, maximum of five indels per locus and a maximum of five low quality sites per read.

2.5. Phylogeographic analysis of population structure

We first analyzed the spatial genetic structure of *F. petiolaris*' molecular data using a genetic clustering method implemented in the R package *conStruct* (Bradburd et al., 2018). This recently developed package uses a method similar to the popular STRUCTURE software where a Bayesian iterative algorithm is employed and individuals with similar patterns of genetic variation (similar ancestry) are placed in a designated number of groups (Pritchard et al., 2000). However, *conStruct* also allows to take the geographic distance of the populations into account. Assuming restricted dispersal dispersal over the geographic scale represented by the samples, genetic differentiation is expected to develop by genetic drift among spatially distant populations due to genetic isolation by distance. Thus, spatially distant versus near populations are expected to display larger genetic differentiation. In cases in which populations are not sampled evenly in geographic space, like the populations of *F. petiolaris* used for this study, genetic clustering in distant populations might be attributed to the distance and not to discrete processes. *conStruct* proposes a spatial method that overcome this problem by calculating an "admixture proportion" which is the contribution of each *K* layer to the ancestry of each sample or population, incorporating decay of that relatedness with distance. We ran *conStruct* for K=1 to K=7 for 100,000 generations in three different chains and performed cross-validation for each *K* with a subsample of the data for 100,000 generations to guide us choosing the most likely *K* genetic groups. This analysis was a first exploration of the data aiming to identify whether *F. petiolaris*' populations clustered within sampling region or genetically behave differently. We also performed a principal component analysis (PCA) to visualize genetic distances between individuals in space. This exploratory analysis complements to the clustering method described above by visualizing how individuals differ genetically from each other and their clustering or

dispersion in PCA space. Finally, phylogenetic relationships among *F. petiolaris*' individuals and the six outgroup species (*F. carica*, *F. sycomorus*, *F. pertusa*, *F. citrifolia*, *F. tonduzii*, *F. insipida* and *F. yoponensis*) were generated using RAxML version 8 (Stamatakis, 2014), which estimates phylogenetic trees and their likelihoods. We searched for trees with maximum likelihood, using the general time reversible model of substitution and a gamma distributed rate variation among sites as nucleotide evolution parameters. We ran 100 distinct maximum likelihood analyses starting from 100 distinct randomized trees and generated bootstrap values as node support. The best tree (i.e., with highest maximum likelihood) was then selected to investigate the relationship of samples within and among populations, and to identify potential phylogroups.

2.6 Geographical patterns of genetic diversity and differentiation

Genetic diversity was calculated as the mean heterozygosity H_s for populations and phylogroups. Northward expansion was investigated by testing H_s for each population against the latitude with linear regressions. Because we were testing two range expansion hypotheses in Baja California and Sonora only, only populations from these regions were tested. We investigated the genetic differentiation among phylogroups as the fixation index F_{st} of populations as in Weir & Cockerham (1984). To identify the portion of genetic differentiation attributable to genetic isolation by distance, we performed a Mantel test (999 iterations) on populations' genetic distance versus geographic distance matrices, both within region and across region. We emphasized especially Baja California samples, because pairs of samples within other phylogroups were limited. Nei's genetic distance was calculated among populations (Nei et

al., 1983) and we used the package geosphere to calculate accurate geographic distances between geolocalized fig trees (Hijmans, 2017).

3. Results

3.1. Past habitat suitability

Results of past suitability modeling where preliminary highlighted in the introduction in order to guide and justify the hypotheses stated. They show a contraction of the suitable habitat for *F. petiolaris* to the south of Mexico during the last glacial maximum (Figure 2) compared to current suitability (Supplemental Figures 2 and 3). The past suitability of *F. petiolaris* was particularly constricted in the cape region in the peninsula of Baja California, while located along the southern and western coast in the mainland.

3.2. Genomic data

137,216,883 single end raw reads were obtained from the sequencer. After *de novo* assembly, 26,529 loci including SNPs were discovered. The step that filtered out most loci was the removal of those found in less than 50% of the samples, which indicates many missing data across individuals, and resulted in 1,409 loci retained. On average, two SNPs were found at each locus. To prevent the use of linked SNPs, only loci with a single SNP were retained for downstream analyses (randomly chosen among loci with the least missing data). We later removed outgroups, replicates and individuals with more than 70% of missing genomic data across loci which revealed many presumed polymorphic loci to be monomorphic across the *F. petiolaris* samples of direct interest. Removing monomorphic loci resulted in a final data set consisting of 727 each loci containing one biallelic SNP.

3.3. Phylogeographic analysis of population genetic structure

The genetic clustering analysis based on ancestry realized with *conStrut* was run for K = 1 to K = 7 and cross-validation revealed significant improvement from K = 1 to K = 2 ($P <$ 0.001), K = 2 to K = 3 ($P < 0.001$), but no significant improvement of the mean predictive accuracy from K = 3 to K = 4 ($P > 0.05$).Thus we present here the spatial genetic structuring for K = 3 (Figure 5). The three genetic clusters identified are as following: one genetic group representing all Baja Californian populations assigned to one genetic cluster (while population 39, 96 and 158 have a small proportion of admixture with other clusters), central Mexico assigned to another genetic cluster and both Oaxaca populations being assigned to the central Mexican cluster and admixed with a third cluster. This suggests some shared ancestry between Oaxacan populations and central Mexico which is nearly absent in Baja California, but because these southern populations have a proportion of admixture assigned to a third cluster, they will be treated as a separate phylogroup. In Sonora however, the pattern is different: two Sonoran populations (100-T and 104) show proportion of admixture intermediate between Baja Californian populations and Central Mexican populations with almost 50% admixture assigned to two of three clusters. Population 103 however, is assigned to the Central Mexican cluster with a small proportion assigned to a third (assigned in Oaxaca populations, cluster green in Figure 5). This first exploration of genotypic distribution in space identifies clearly three phylogroups: Baja California, central Mexico, and Oaxaca, with Oaxaca always admixed with another unsampled genetic group. The situation in Sonora is more complex however, with population 103 clearly different than coastal populations (100-T and 104). Because these populations show differences in ancestry, they cannot be considered distinctive uniform group. Thus, coastal populations

100-T and 104 are assigned to their own phylogroup (coastal Sonora) and inland Sonora population 103 is assigned to central Mexico based on its genetic similarity.

The two principal components of the PCA with the largest eigenvalues explained most of the variation in the PCA space. They are thus the only ones that will be presented here in a two-dimensional plot (Figure 6). Genetic differences among individuals clustered space support the preceding analyses with Baja Californian population forming an aggregated group of short genetic distances (dark blue) relative to other groups. The central Mexican individuals form a group (red), but with larger genetic differentiation among individuals than within Baja California (i.e., points in space farther apart). The Oaxacan individuals form another clearly differentiated genetic group (light blue), also with a larger degree of differentiation between populations than Baja California. As seen previously, coastal Sonoran samples are spread between the groups of Baja California and central Mexico groups. Similarly, inland Sonoran samples project in space with samples of central Mexico but we note two samples which that projected towards coastal Sonora samples. The contrasting result in this PCA with the clustering methods is that the Oaxacan samples are clearly discriminated from the other groups, a result which was more ambiguous in the ancestry clusters and will be regarded with caution. With this information we have evidence of genetic structure between Baja California, Central Mexico and Oaxaca. Sonoran populations although geographically near exhibit different genetic compositions with each other. Coastal and inland populations again clearly show strong differentiation within Sonora.

The *F. petiolaris* phenogram obtained with RAxML showed in general low support in bootstrap values (Figure 7). However, the monophyly of the individuals of Oaxaca was highly supported (100%, not visible on the figure). High (>70%) support values were also observed in

some rare instances at some nodes connecting *F. petiolaris* individuals from the same population. These values, which indicate low support of phylogenetic relationships between the majority of the samples suggest some low structure between samples. However, in this analysis, most individuals are from Baja California, which form a large clade with no structure within (i.e., individuals are not grouped by populations). On the other hand, individuals from Oaxaca form a monophyletic, sister group to central Mexican samples, nested among individuals of central Mexico and Sonora with moderate support (69%). Sonora samples are dispersed in the phenogram: most of the individuals form a paraphyletic group at the base of the branches from individuals from Baja California, but some are nested within the large Baja Californian clade. Individuals from population 103 however are sister to sample from central Mexico, as well as some individuals from population 104. Conversely, Sonoran samples show polyphyly. Because of the low bootstrap support values, the phylogeny does not provide robust information and should be interpreted with caution. However, the information obtained from the tree supports the conclusions made above: we distinguish three phylogroups (Baja California, Oaxaca and central Mexico) and Sonoran samples show either intermediacy between central Mexico and Baja California (with some individuals within the Baja California clade) or are related to central Mexican samples (population 103). Thus, from the phylogenetic tree and the clustering analysis, there is evidence for four phylogroups with coastal populations (100-T and 104) which show intermediacy between Baja California and central Mexico and population 103 located in Sonoran clustered with central Mexico.

3.4. Geographical population genetics

In Baja California, genetic diversity within populations (average expected heterozygosity was H_s = 0.051, which is lower than observed for the other three phylogroups. In mainland Mexico, expected heterozygosity increased with decreasing latitude of the phylogroup: coastal Sonora mean H_s = 0.089, central Mexico mean H_s = 0.148, and Oaxaca mean H_s = 0.165. Genetic differentiation among phylogroups was high (0.1399 – 0.6590; Table 2). Higher genetic differentiation was observed between Baja California and Oaxaca, which are the most distant groups of populations, while the smallest was between coastal Sonora and central Mexico. We note that although population 103 occurs in Sonora it was assigned to central Mexico phylogroup based on genetic clustering, which could contribute to the lower value of F_{st} between the coastal Sonora and central Mexico phylogroups (Table 2).

Population genetic diversity relative to the latitude of the populations can be seen in Figure 8. Population heterozygosity follows the same trend as presented above with central Mexico and Oaxaca (mainland Mexico on the figure) with higher genetic diversity than coastal Sonora and Baja California. All population heterozygosity means were low in Baja California but population 103 showed a significantly higher level than others (Figure 8). Interestingly, the populations of coastal Sonora show roughly intermediate values of population genetic diversity among the five phylogroups. The relationship between population genetic diversity and latitude tested with linear regressions was not significant in mainland Mexico (coastal Sonora excluded) (ß= -0.0015, P = 0.23) or in Baja California (ß= 0.0004, P = 0.62), thus providing no evidence for lower genetic diversity in populations at higher latitudes.

Because samples of mainland populations were sparse, testing for isolation by distance was conducted for Baja California samples only. Figure 9 presents the genetic distances between

pairs of populations within the peninsula against their geographic distances. A moderate but significant positive trend was observed ($r = 0.278$, $P = 0.047$) indicating that genetic differences between distant populations were small, but significantly larger than differences between nearby populations. This suggests that dispersal is slightly limited, allowing for some genetic differentiation in distant populations through genetic drift.

4. Discussion

Glacial oscillations of the late Pleistocene led to shifts of habitat along the latitude causing important changes in species distribution (Hewitt, 2003). Species' ranges were constricted toward the equator during glacial maxima and followed with post-glacial colonization toward the poles (Hewitt, 1999). Range constriction into refugia lead to reduction of genetic diversity (Arenas et al., 2011) and post-glacial colonization is expected to create patterns of genetic diversity loss along the axis of the expansion and isolation by distance (Hewitt, 1996). Furthermore, populations constricted by physiographic barriers could experience population bottlenecks which could also result in loss of genetic diversity prior to post glacial re-colonization (Allendorf, 1986). Alternatively, complex genetic structure could arise from colonization from multiple glacial refugia and/or effects of long-distance colonization (Ibrahim et al., 1996).

In Baja California, genetic clustering analysis of individuals sampled from 12 locations indicated all belong to a single genetic group, except three populations (158, 39 and 96) displaying very low amounts of admixture (Figure 5). The relatively low genetic distances between these individuals (Figure 6) and the complete lack of resolution observed in the best maximum-likelihood phylogenetic tree indicate no population genetic structure within Baja

California, thus suggesting genetic connectivity between all these populations. Connectivity could be due to pollen dispersal by fig wasps or seed dispersal by animals within Baja California. Mean population heterozygosity was also the lowest in Baja California, relative to other populations of other phylogroup (Figure 8). These results provide evidence for low genetic diversity in Baja California possibly associated with a genetic bottleneck. This could be attributed to a range constriction to a single glacial refugium in the cape region to the south of the peninsula, as suggested by the past habitat suitability projection inferred (Figure 2), or alternatively to a recent long-distance colonization event from the mainland to Baja California with founder effect. Baja California has a high level of genetic differentiation from phylogroups on mainland Mexico, suggesting that these populations have been isolated for a long-time and that such a long-distance colonization event from mainland to Baja California would have to have occurred before the late Pleistocene period. We conclude that *F. petiolaris* probably experienced a population bottleneck in Baja California that led to low genetic diversity observed but there is however little evidence for a post-glacial expansion from south to north in the peninsula. Heterozygosity showed no significant loss at higher latitude but moderate isolation by distance was observed. It is intriguing that the population that showed larger genetic diversity in Baja California is not located in the cap region but in the middle of the range of *F. petiolaris* in the peninsula (population 113, figure 8). This might indicate a potential refugium at a mid-peninsular latitude rather than in the south, as seen in other plants (Garrick et al., 2009). However, past habitat suitability does not support this scenario (Figure 2). Finally, because we believe that shallow-time demographics have influenced the current spatial genetic distribution of populations in Baja California, we did not expected a signature of historical vicariances due to a potential Isthmus of La Paz, a mid-peninsular seaway, nor a break located around Loretto.

These events were not supported by the genetic data. This adds another evidence that plants are more sensitive to past climate fluctuations than animals, with disturbance in their distributional range.

From ancestry assignment and PCA, we identified two additional obvious genetic clusters associated with their geographic location: central Mexico and Oaxaca. Past habitat suitability modeling show that the range of *F. petiolaris* might have shifted toward the southern coast of Mexico but that populations were likely connected. Indeed, the suitability analysis showed no discontinuity along this potential past range. The Oaxaca region probably provided suitable habitat during the LGM, but nearby central Mexican populations are located outside the predicted LGM suitability range. This means that in Oaxaca, populations likely were not disturbed by the LGM climatic oscillation and thus have preserved large genetic diversity. The high levels of mean population heterozygosity observed in the Oaxaca samples compared to those taken farther north in our study are concordant with this hypothesis. Central Mexico was likely colonized from this large nearby ancestral population, and therefor also retain relatively high levels of genetic diversity. Because of the large spatial discontinuity between the populations studied in mainland Mexico, it is hard to distinguish if the genetic differentiation observed between both regions is due to genetic drift, or if large physiographic barriers have contributed to vicariance between both regions and we can't draw conclusions at the time. Additional populations sampling and analyzing is necessary to resolve this question.

In Sonora, genetic patterns differ between populations. Population 103 is clearly genetically similar to populations of central Mexico. This population, which was probably colonized after the LGM likely its was founded from populations at the limit of the northern range during the last ice age. Individuals from this limit (current central Sinaloa) probably were

not isolated from the rest of *F. petiolaris* and carried greater genetic diversity than populations in Baja California that experienced a bottleneck. If so, it is not surprising that the mean expected heterozygosity in Sonora is similar to that in central Mexico. We expected, however, that populations 103 and in central Mexico (populations 215 and 217) to have lower level of genetic diversity than Oaxaca, because Oaxacan populations seem to have been located in, or near, the southern refugium of central Mexico. (Figure 8). It is also striking that population 103 has a higher level of genetic diversity than the central Mexican populations because 130 is further away from the probable past distribution of *F. petiolaris.* We recognize however that the number of populations sampled in Mexico is small and additional populations are necessary to draw conclusions regarding patterns of genetic diversity in central Mexico.

Populations 100-T and 104 located near the coast of the Sea of Cortez showed an obvious genetic signature of admixture with Baja California populations. Even though the Sea of Cortez represents an important physiographic barrier of several hundreds of kilometers, our data suggest that Sea of Cortez does constitute a source of vicariance (F_{ST} value is larger between coastal Sonora and Baja Californina than between more distant populations within Baja California), but is not an absolute barrier to dispersal between the mainland and the peninsula. The dispersal also seems unidirectional with gene carried from Baja California to Sonora. Garrick et al. found a similar connectivity with *Euphorbia lomelii*. The distribution of this Sonoran Desert euphorb is located mainly in Baja California, in the desert of Sonora and north of Sinaloa. The authors suggest that seed dispersion occurred from Baja California to the desert of Sonora which was associated with a reduction of genetic diversity. Here we are not concerned about colonization events from Baja California to the mainland but about dispersion of pollen by female fig wasps or seed dispersal by avian vertebrates. Fig wasps are good dispersers and Nason et al. (1998)

showed using genetics that they can likely travel hundreds of kilometers. The wind in the Sea of Cortez is on average following a south-east direction with winds usually stronger in Baja California then on the Sonoran coast (Parés-Sierra et al., 2003). This is consistent with probable dispersal events from Baja California to the mainland. North-west winds also occur and pollen dispersal from mainland to Baja California could also be possible (Parés-Sierra et al., 2003). However, our data argue against dispersal in this direction, or that is very limited and could explain the small proportion of admixture, only observed in populations 39, 96 and 158. An alternative to this explanation could be dispersal of seeds by avian vertebrates like bats. Seeds in *Ficus*' fleshy multiple fruits are generally dispersed by mammals or birds (Shanahan et al., 2001). Bats are particularly well known to feed on mature figs (Morrison, 1978; Kalko et al., 1996; Korine et al., 2000). In western Mexico, *Leptonycteris curasoae* has a range that covers Baja California and Sonora and travels long distance (Wilkinson & Fleming, 1996). They may contribute to *F. petiolaris* seed dispersal. However, the authors argue that migration by bats between these two regions is rare.

Interestingly, the admixture observed in coastal Sonora populations is absent in population 103 (inland Sonora) which is 244 km and 90 km away from populations 100-T and 104, respectively, but located in mountainous terrain (Figure 2). This may suggest that cross-sea pollen dispersal is recent (i.e., F1 offspring) and trees from coastal populations have not been outcrossing with trees of population 103 yet, or that gene flow is restricted between coastal and inland populations. The latter scenario would be surprising, though, because we do not know of any physiographic barrier in the region. An alternative could be that both coastal and inland Sonoran populations may have been colonized from different isolated refugia and experienced post-glacial secondary contact while undergoing genetic differentiation, resulting in nearby

populations with high genetic differentiation. This seems unlikely, however, because our species distribution modeling under late Pleistocene climates did not identify isolated refugia. It seems more likely that the observed differentiation is due to genetic admixture from Baja California. Finally, we notice that some coastal individuals share high genetic similarity with individuals from populations 39 and 112 (Figure 7). These could be direct offspring from seeds transported from Baja California to Sonora by avian endoallochory, suggesting that dispersion by bats or other avian vertebrates might be more common than previously thought.

Together, our results suggest that shallow time historical range contraction and expansion are responsible for the genetic landscape of *F. petiolaris* in Baja California and Sonora, and we reject hypotheses of deep time historical vicariance. Fig trees in Baja California likely experienced a population bottleneck in one refugium. However, we found no evidence for post-glacial northward expansion, in contrast to strong evidences for northward expansions in columnar cacti (Nason et al., 2002; Clark-Tapia & Molina-Freaner, 2003). Based on genetic clustering results and the pairwise F_{ST} value between Sonora and Baja California, it appears that the Sea of Cortez represents an important physiographic barrier to gene flow. This barrier is, however, not completely impassable, with evidence of dispersal from Baja California to Sonora with the presence of admixed populations along the coast. In mainland Mexico our conclusions are less assured. We notice large differentiation between coastal Sonora, central Mexico and Oaxaca but can't affirm that this is due to historical vicariance caused by the major physiographic barriers that separates them. However, the genetic proximity of population 103 to central Mexican populations, consistent with a northward expansion of *F. petiolaris* from ancestral southern population.

5. Conclusion

Historical vicariance has played an important role in shaping the genetic diversity of many taxa in Mexico (Riddle et al. 2000; Riddle & Hafner, 2006; Dolby et al., 2015). However genetic patterns in plants are structured by more recent historical demographic events associated with paleoclimatic fluctuations (Nason et al., 2002; Clark-Tapia & Molina-Freaner, 2003; Garrick et al. 2009). Here we bring another piece of evidence that shallow time climatic events affected the biogeography and phylogeography of a plant species, *F. petiolaris*, along the Gulf of California. Patterns in mainland Mexico, however, must be interpreted cautiously until additional sampling provides a better description of the genetic landscape in these regions.

It has been recommended in the literature to combine approaches (i.e., fossil records, phylogeography and species distribution modeling) to obtain robust insights of historical processes in species, notably for glacial refugia detection (Gavin et al., 2014). We used species distribution modeling prior to determination of geographic genetic structuring to guide robust hypothesis generation and *a posteriori* hypothesis testing. Here, the presence of a small suitable habitat in the cape region of the Baja California peninsula, as well as extensive coastal mainland habitat during the LGM helped us formulate and test shallow-time hypotheses over historical vicariance to reconstruct the history behind current genetic structure.

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Table 1. *Ficus petiolaris* study populations sampled for phylogeographic analysis.

Region	Population	Latitude	Longitude	Sampled trees
Baja California	158	29.26359723	-114.0216665	16
	172	28.29038826	-113.1110026	16
	112	27.56491059	-113.0711841	19
	113	27.0995915	-112.4968451	15
	95	26.35797318	-111.8027891	16
	179	25.913455	-111.349716	19
	201	25.38127688	-111.3151591	19
	204	24.82815902	-110.8077579	6
	96	24.03565692	-110.1232369	13
	70	23.7377989	-109.8303991	15
	39	23.13640033	-109.772005	7
	205	23.04974805	-110.0626406	7
Sonora	100-T	27.94426216	-111.0843175	5
	104	27.13673531	-109.769192	6
	103	26.94244195	-108.8812348	4
Central Mexico	217	21.20072032	-103.188336	6
	215	20.75226385	-103.3229411	6
Oaxaca	214	16.63305268	-96.05774833	5
	210	16.39167118	-95.38354844	3

Table 2. Multilocus estimates of pairwise *Fst* between inferred phylogroups of *F. petiolaris.* Significance: *** = $P < 0.001$. Values in parentheses are bootstrapped 95% confidence intervals.

	Baja California	Coastal Sonora	Central Mexico	Oaxaca
Baja California	NA			
Coastal Sonora	0.2466*** (0.1985–0.2928)	NA		
Central Mexico	0.5297*** (0.4749–0.5800)	0.1399*** (0.1010–0.1835)	NA	
Oaxaca	0.6590*** (0.6133–0.6922)	0.3789*** (0.3330–0.4030)	0.2784*** (0.2258–0.3012)	NA

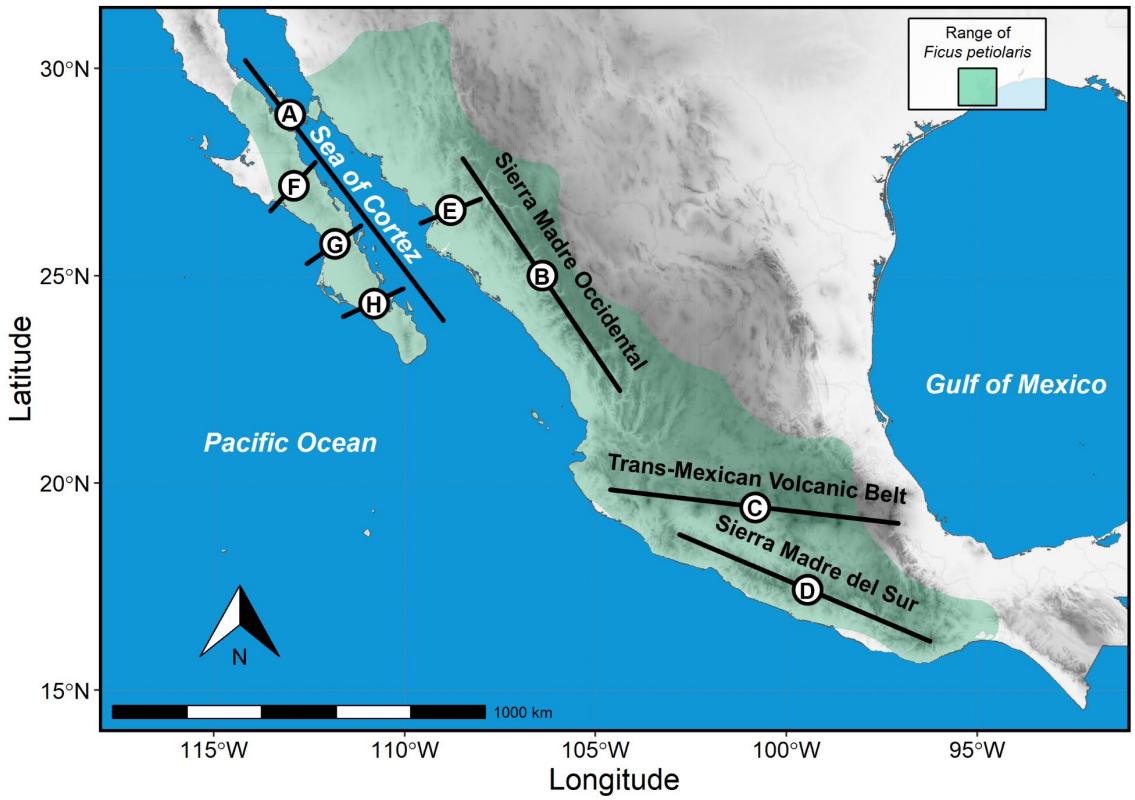

Figure 1. *A priori* vicariance hypotheses for *F. petiolaris* based on past and current physiographic barriers. (A) Sea of Cortez (ca. 5 Myr), (B) Sierra Madre Occidental (ca. 38-20 Myr), (C) Trans-Mexican Volcanic Belt (ca. 11-3 Myr), (D) Sierra Madre del Sur (ca. 48-23 Myr), (E) Sierra Barabampo-Rio Fuerte break, (F) mid-peninsular seaway (ca. 1 Myr), (G) Loreto break and (H) Isthmus of La Paz (ca. 3 Myr).

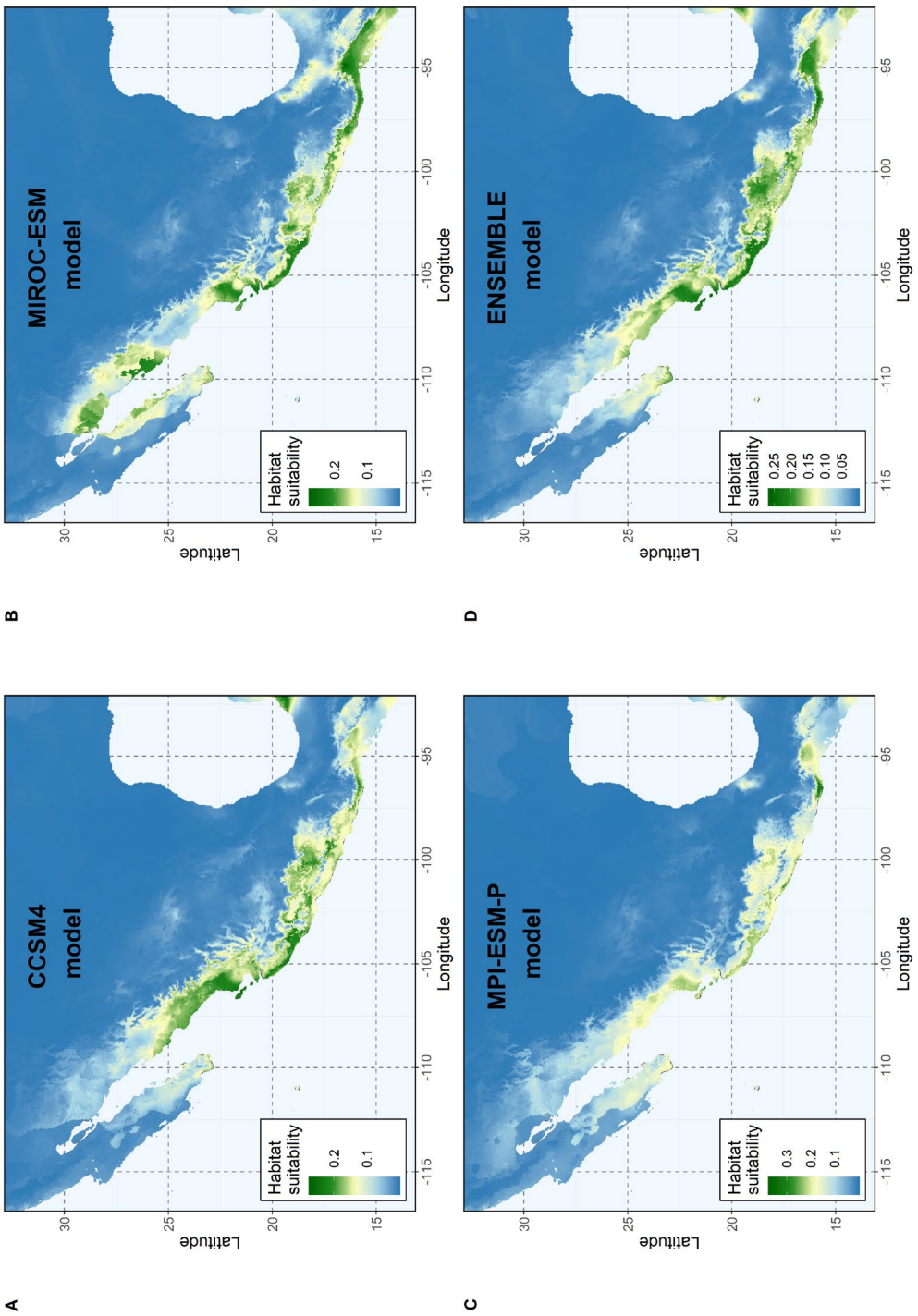

Figure 2. Habitat suitability for *F. petiolaris* projected under paleoclimatic conditions at the LGM (worldclim data). Projections were realized with a weighted model combining all the model trained with current climate data, for past scenarios. A) CCSM4 scenario, B) MIROC-ESM scenario, C) MPI-ESM-P scenario, and D) EMSEMBLE averaged scenario.

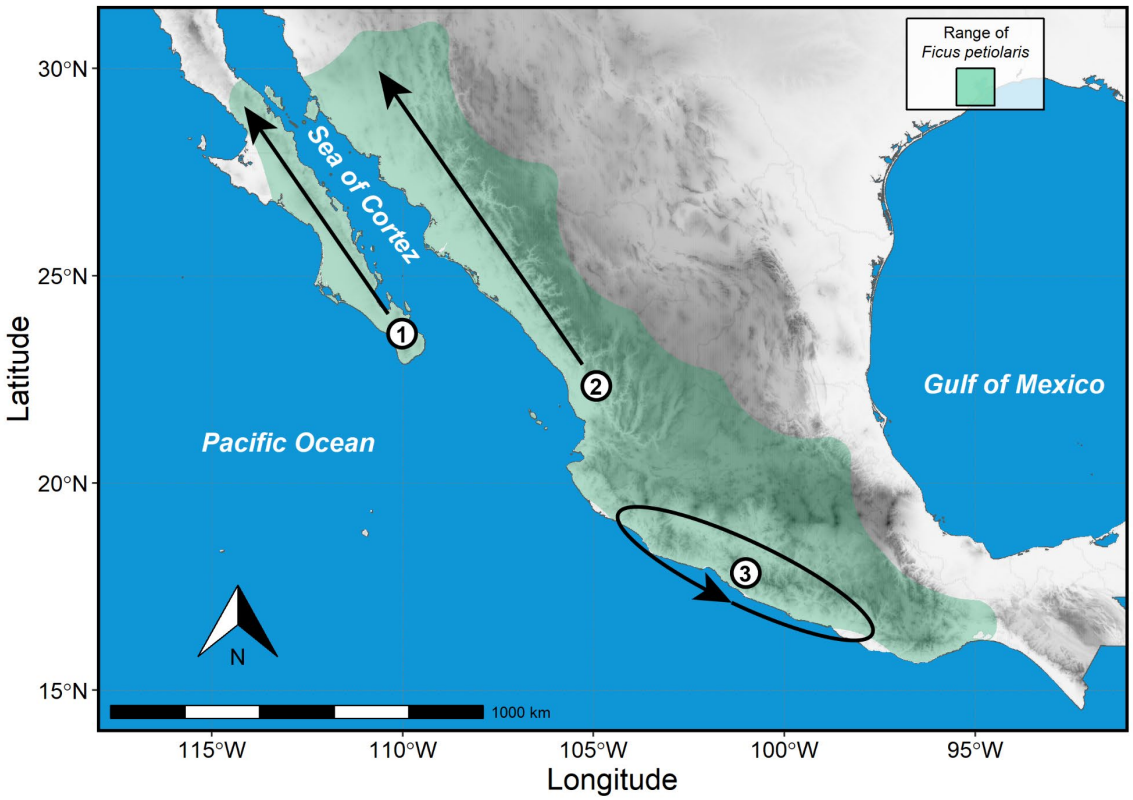

Figure 3. Post-Pleistocene changes in the distribution of *F. petiolaris* predicted *a posteriori* from hind-cast niche modeling. (1) Northward range expansion from the Cape Region of Baja California and (2) northward range expansion in northwestern mainland Mexico from the southern limit of habitat suitability in this region during the LGM. In addition, we predict (3) the continued maintenance of coastal populations in southern Mexico from the LGM to the present.

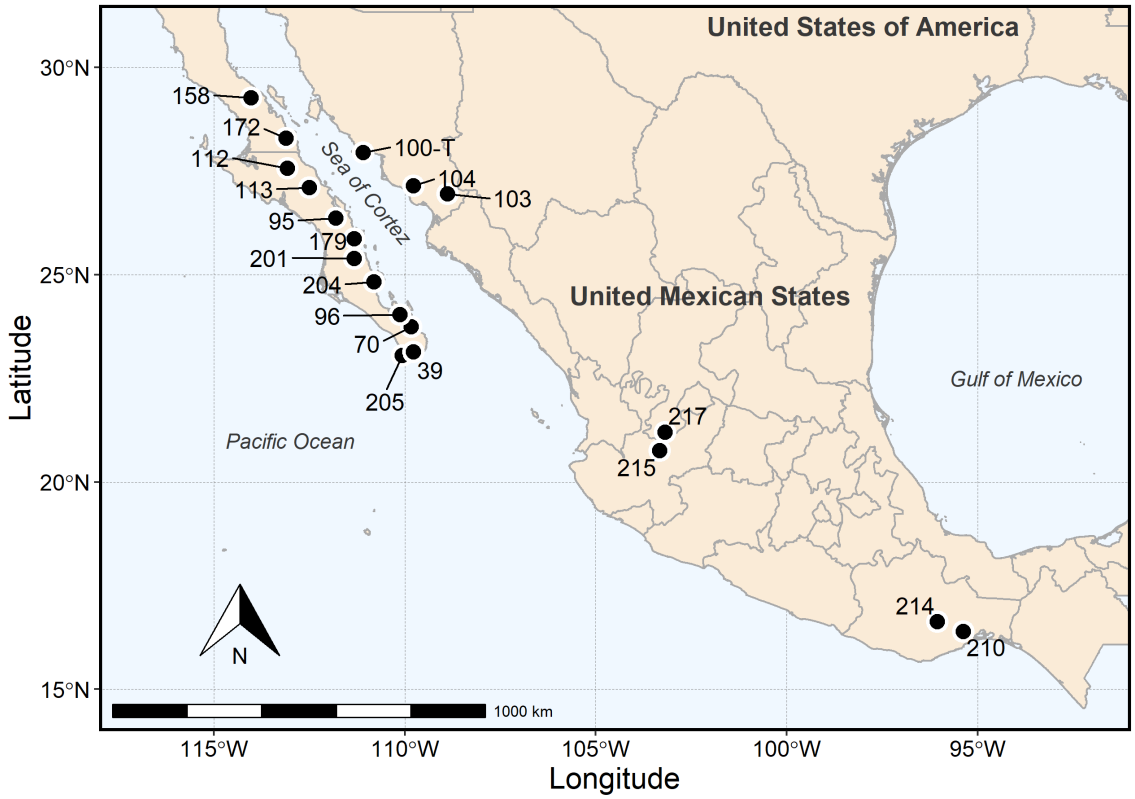

Figure 4. *Ficus petiolaris* populations sampled for this study.Four regional groupings are represented: Baja California (populations 158, 172, 112, 113, 95, 179, 201, 204, 96, 70, 39 and 205), Sonora (populations 100-T, 104 and 103), central Mexico (populations 217 and 215), and Oaxaca (populations 214 and 210).

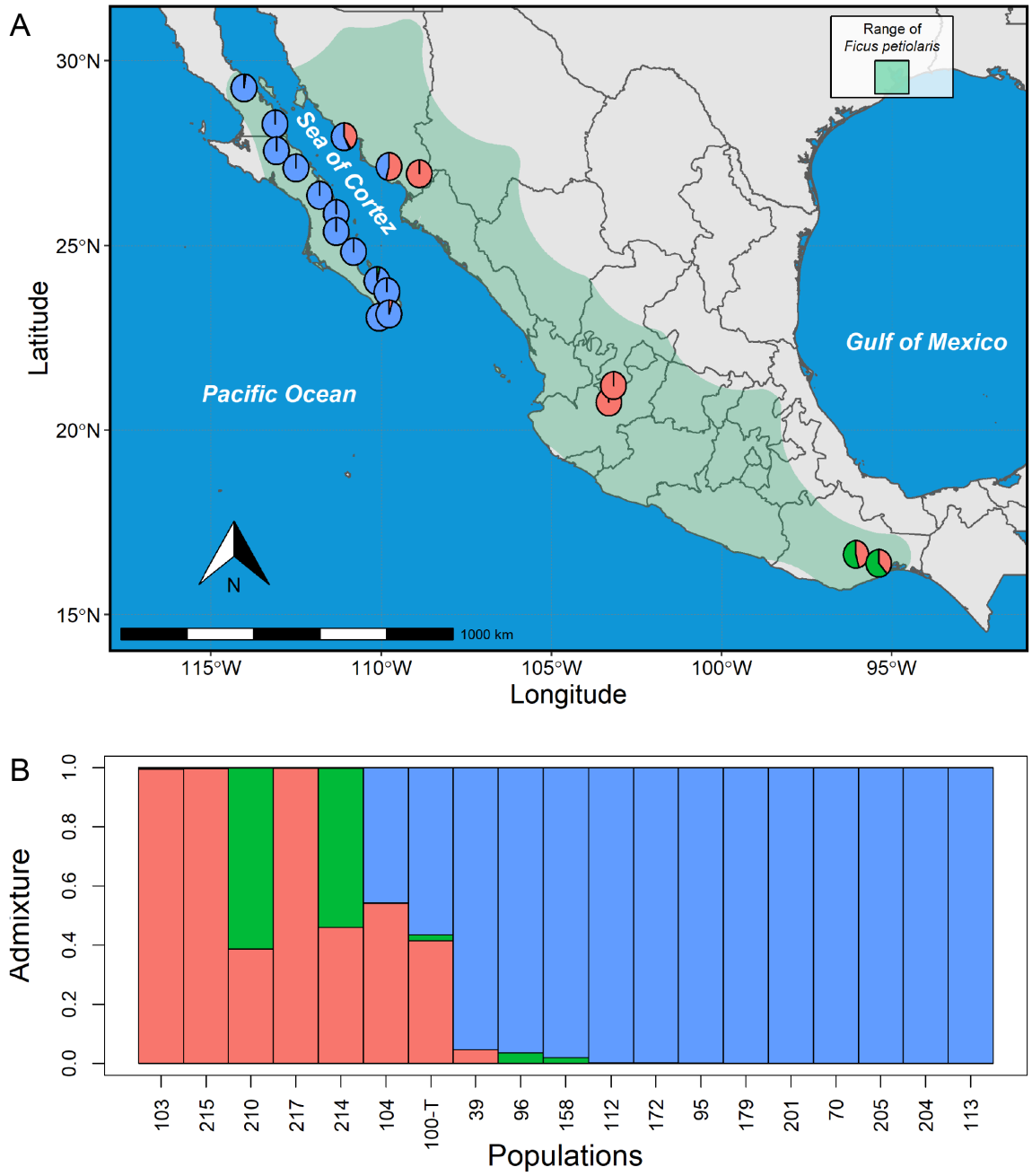

Figure 5. Spatial population genetic clustering analysis of *F. petiolaris* conducted with *conStruct.* for the optimal value of $K = 3$ identified from cross-validation.The three clusters are colored blue, red, and green and the proportions of individuals assigned to these clusters are represented as A) pies charts mapped on the locations of *F. petiolaris* sample populations, and B) a stacked bar chart indicating population designations. Geographic correspondence to population designations are in Figure 4.

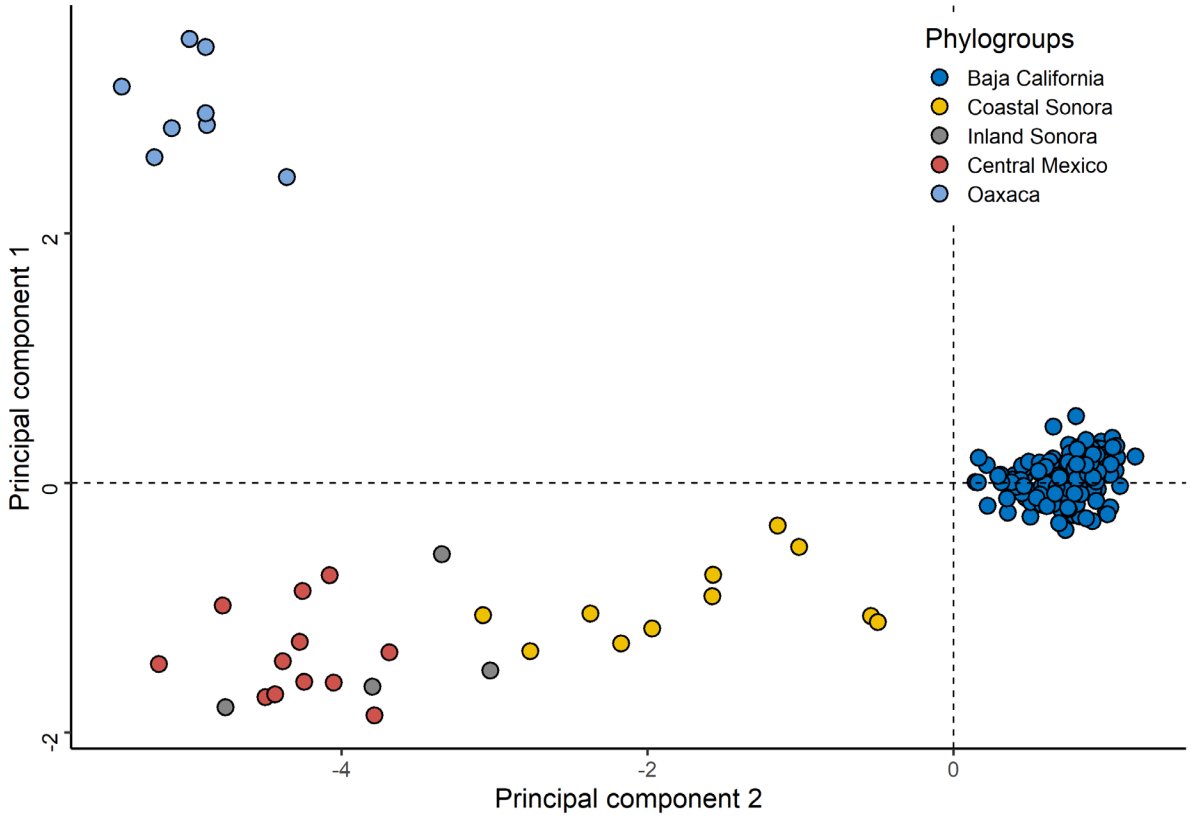

Figure 6. Principal component analysis of genetic diversity of *F. petiolaris*' individuals. We identified three genetic groups (Baja California, central Mexico and Oaxaca, dark blue, red and light blue respectively) and one admixed group (coastal Sonora). We highlighted inland Sonora (population 103) in gray because it is genetically clustered with central Mexico but is geographically located in Sonora for better interpretation.

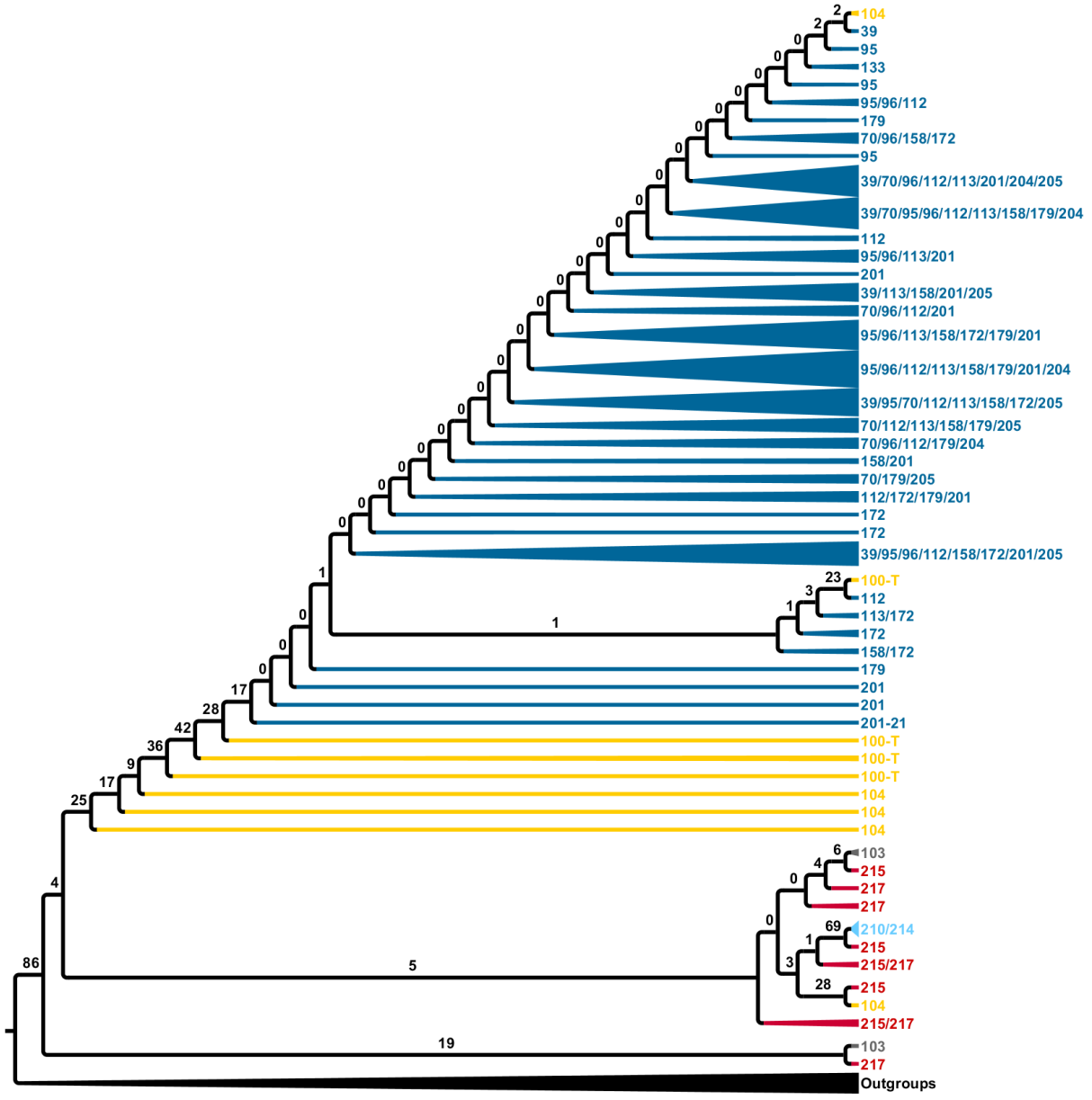

Figure 7. Maximum likelihood phylogenetic tree of *F. petiolaris* populations. Colors represent regional phylogroups identified in previous analyses and are the same as in Figure 5. Monophyletic groups are collapsed into individual branches for visibility. Width of the branches represent the proportion of individuals grouped within branches, their populations is indicated at the tips. Node values represent bootstrap support (not shown in collapsed node). Outgroups are: *F. carica*, *F. sycomorus*, *F. pertusa*, *F. citrifolia*, *F. tonduzii*, *F. insipida*, *F. yoponensis*.

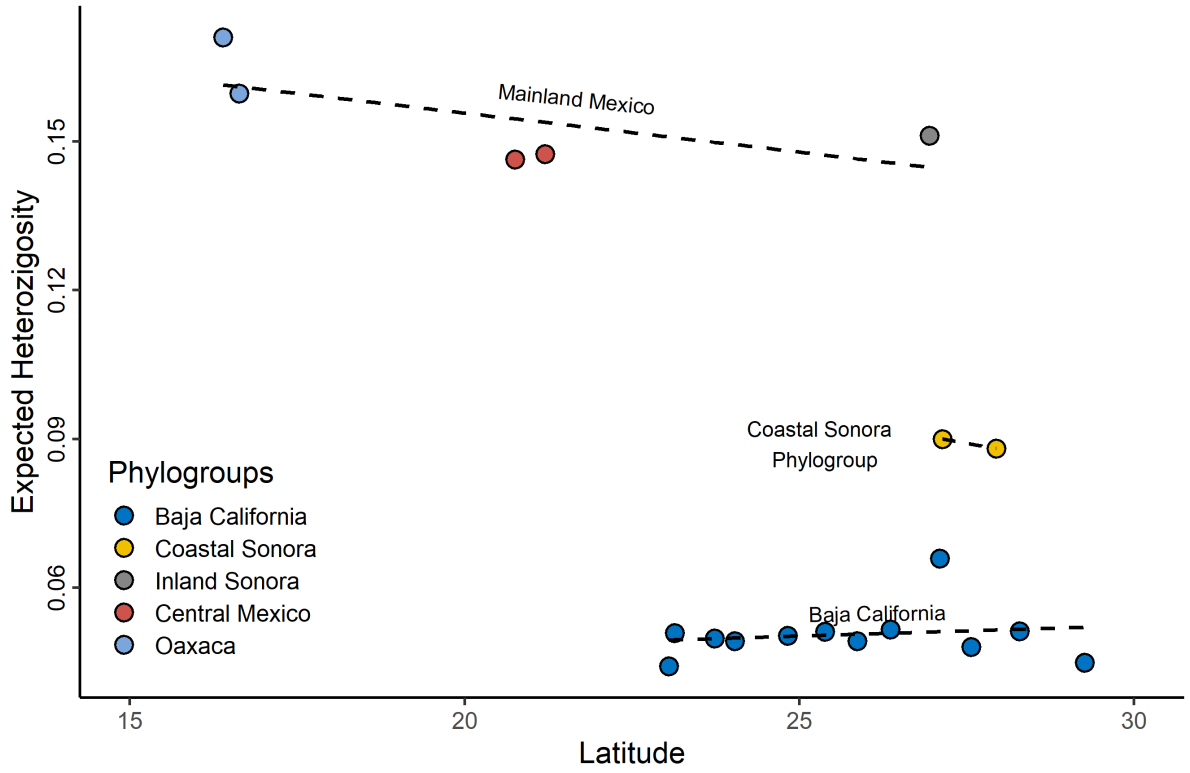

Figure 8. Expected heterozygosity of *F. petiolaris* populations as a function of latitude. Colors are as in Figure 6 and represent regional phylogroups identified from genetic analyses. The Baja California population with the highest heterozygosity is population 113 (see Table 1).

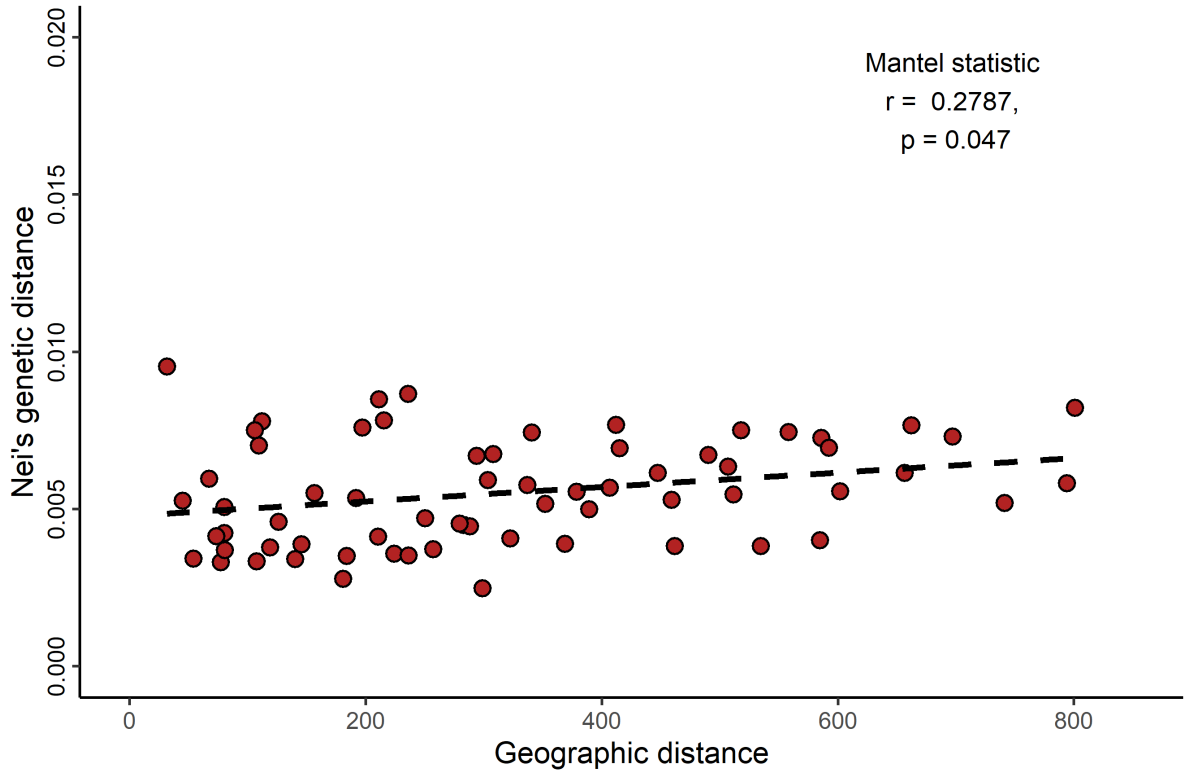

Figure 9. Isolation by distance in Baja California. Points represent Nei's genetic distance between pairs of populations within Baja California relative to the pairwise geographic distance separating them (in km). The Mantel test is based on 999 permutations.

CHAPTER 3. LANDSCAPE-LEVEL ANALYSIS OF A FIG-POLLINATOR-PARASITE COMMUNITY: GEOGRAPHIC AND TEMPORAL VARIATION IN SPECIES REPONSES TO BIOLOGICAL AND CLIMATIC VARIABLES

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Abstract

Flowering plants are ubiquitously associated with diverse assemblages of insects. Interactions vary from beneficial to antagonistic, and the dynamics of these interactions can be influenced by variations in their environment. In the present study, we investigate the combined effects of several ecological factors on a fig-fig wasp mutualism and their insect antagonist dynamics. *Ficus petiolaris* is a fig trees that grows on rocks in the arid Sonoran Desert (Mexico). It is associated with an obligate pollinating wasp and host-specific antagonist non-pollinating wasps. Unlike most fig species, *F. petiolaris*' populations are small, patchy and dense, and it produces relatively small crops of syconia asynchronously within trees. Little is known about how geographic and temporal variation in the wasp community composition, host-related reproductive variables and the abiotic environment affect the fig wasp community associated with fig trees. We visited nine *F. petiolaris* populations four times between late 2012 and late 2014 and measured syconia volume, foundress wasp count, tree volume, reproductive effort,

asynchrony, syconium landscape, temperature and precipitation. We used mixed models to determine the effect of these variables on species counts. Because our ultimate goal is to understand the effect of spatiotemporal variation of the environment on the fig wasp community, we used logistic regressions tested to test the relationship of these variables with the proportion of pollinators produced relative to the proportion of non-pollinating wasps. The results indicate high variation within predictor and response variables and show differential response of wasp species to variation in the environment. Associations between wasp counts were also tested for significance and deductions about their biology are discussed. Major findings are that within-tree asynchrony benefits the non-pollinating fig wasps more than the pollinators and that increased temperature and precipitation favors pollinators over non-pollinators. We argue that observational fig wasp community studies should integrate spatial and temporal perspectives into their design, and we advise caution when interpreting the dynamics of species communities in a single environmental context.

1. Introduction

Plants, primary producers essential to most ecosystem functioning, are often involved in mutualistic interactions (Bronstein et al., 2006). In fact, the enormous diversification of flowering plants during the lower Cretaceous appears to be due to a confluence of factors, including their remarkable coevolution with animal pollinators (Lunau, 2004; Hu et al., 2007; Crepet and Niklas, 2009). Today, nearly 85% of land plants are angiosperms and, of the estimated 352,000 extant species (Paton et al., 2008), 85% are pollinated by animals (Ollerton et al., 2011). In these mutualisms, plants are provided with pollination and pollen dispersal essential to reproduction while most pollinators, mostly insects, are rewarded with resources.

These interactions, among the most studied mutualisms (Bronstein, 1994) are ubiquitous and provide important ecological services for human agriculture (Boucher, 1985), and contribute to ecological community structure and maintenance of biodiversity (Stachowicz 2001; Bascompte et al., 2006). However, mutualistic partners each can have very different biologies, and spatial and temporal variation in biotic or abiotic factors can potentially differentially affect them, resulting in geographic variation in species interactions and outcomes (Thompson, 1988; Travis, 1996; Chamberlain et al., 2014; Trojelsgaard & Olesen, 2016) that can shift through time (Holzapfel & Mahall, 1999). The ecological and evolutionary responses of mutualisms to the environment, including to anthropogenic threats, can be diverse (Bronstein et al., 2014). Despite our large knowledge of plant–pollinator interactions, gaps remain, and we lack understanding of their dynamics and persistence in a spatial, temporal and community context, which are essential to predicting their responses in the current context of rapid global change.

Many, perhaps all mutualisms, including plant–pollinator interactions, are subject to exploitation (Bronstein, 2001). In fact, mutualisms occur within complex communities in which species interact in various ways along a mutualism-antagonism continuum (Bronstein, 1994). Variation in interaction of one or several species can potentially affect the dynamics of a species' entire interaction network. As for mutualists, variation in the biotic and abiotic environments of individual species' responses can change the nature of their interactions with all the other species they associate with (Tylianakis et al., 2008), potentially altering the fate of mutualisms (Kiers et al., 2011) and even leading to co-extinctions (Dunn et al., 2009). Because contemporary environmental change is both rapid and widespread, species interactions may be altered in ways that impact global biodiversity and have dramatic ecological and economic consequences (Díaz et al., 2006; Cardinale et al., 2012).

Figs (*Ficus* Kunth*,* Family: Moraceae, 750+ Species, Berg, 1989) and their associated pollinating fig-wasp are a well-known models of nursery pollination mutualism: all fig plants produce syconia (an enclosed inflorescence) typically pollinated by a small (2-3 mm) and short-lived (ca. 2days) host-specific fig wasp that lays eggs in a subset of the fig tree's female flowers. This interaction provides net benefits to the fig through the production of seeds and pollinator wasp offspring that will disperse the fig's pollen. The offspring of the pollinator develop in galled flowers and later emerge and mate. The female wasps collect pollen from newly mature male flowers inside the natal fig, exit, and disperse to find receptive figs on a new host. Fig wasp pollinators are short-lived, requiring the host fig species to produce syconia year-round. This results in flowering phenology that is asynchronous among trees at the population level and that provides fruits year-round for mostly vertebrate frugivores. In addition to pollinators, all fig species are typically associated with host-specific non-pollinating wasps (Bouček, 1993; Weiblen, 2002; Kjellberg et al., 2015). Although phylogenetically diverse, all non-pollinating fig wasps oviposit from outside the syconium and insert eggs into flowers, the syconium wall, or, if parasitoids, into other developing wasps (Borges, 2015). These non-pollinating wasps may negatively impact the fitness of the mutualists by decreasing seed and pollinator production through multiple mechanisms (West & Herre, 1996; Cardona et al., 2013; Conchou et al., 2014; Castro et al., 2015). Although usually less abundant than the pollinating fig wasps (Karunaratne, 2009), spatial and temporal dynamics in the relative abundances of pollinating and non-pollinating fig wasp species are not well studied (Bronstein, 1992; Wang et al., 2012).

Insect behavior and population biology are strongly influenced by their environment (Khaliq et al., 2014), and insects with similar biologies can be differentially affected (Staley et al., 2006). The population biology of fig wasp communities is poorly understood, however,

studies have identified ecological factors influencing their structure and composition. At the individual fig level, syconium size (Cook & Power 1996) and wall thickness (Bronstein, 2001; Zhen et al., 2005; Tzeng et al., 2014) have been associated with increased production of pollinators. Greater numbers of foundresses (female pollinating wasps entering a syconium) also increase total wasp offspring production per fig, but decrease the average reproductive success of individual foundresses (Herre, 1989; Duthie et al. 2016). Host fig population density also differentially affects non-pollinating fig wasp abundance, with poorer dispersers (high wing loading) benefiting from higher host densities, and better dispersers (low wing loading) being relatively more abundant at lower host densities (Duthie et al., 2016). Although syconia typically develop in synchrony within fig plants, within-plant asynchrony occurs in several *Ficus* species (Bronstein, 1989; Bronstein & Patel, 1992; Cook & Power, 1996; Smith & Bronstein, 1996; Gates & Nason, 2012; Piatscheck et al., 2018), and may benefit populations of pollinators and non-pollinating fig wasps by enabling them to disperse and reproduce within the same host plant. It has been suggested (Janzen, 1979; Cook & Power, 1996) that cycling of pollinators within asynchronous fig plants helps to maintain wasp populations and provides reproductive assurance to the fig. However, Gates & Nason (2012) provided evidence that inbreeding depression and abortion of syconia resulting from self-pollination generates strong selection against within-host dispersal of pollinators. They argued that benefits to flowering asynchrony lie in increasing overlap in reproductive phenologies and outcrossing between trees, though at potential costs to benefiting non-pollinating fig wasp populations. Plant population sizes and density differentially affect visitation by pollinator species (Kunin, 1997; Molano-Flores & Hendrix, 1999; Mustajärvi et al., 2001; Bernhardt et al., 2008; Dauber et al., 2010), and benefit pollinator dispersal and short dispersing non-pollinating fig wasps (Duthie et al., 2015; 2016). However, density does not

reflect the landscape of syconia available during oviposition by fig wasps, and the effect of flowering neighboring trees on fig wasp dynamics has no yet been investigated.

Adult fig wasps have a short lifespan and are 1–2 mm long. Past studies have demonstrated the sensitivity of pollinating and non-pollinating fig wasps to high naturally occurring temperature with 50% reduction of their lifespan at approximatively 33°C and near lethality at approximatively 38°C (Patiño et al., 1994; Jevanandam et al., 2013; Khalaf et al., 2015; Sutton et al., 2018). Patiño et al. (1994) showed that transpiration reduces syconium temperature 3-8°C, potentially benefiting pollinator wasps which enter the syconia, at higher temperature, relative to non-pollinating fig wasps, which do not.

All these biotic and abiotic environmental factors that influence the multidimensional niche of fig wasps (Hutchinson, 1957) have differential effects on the reproductive dynamics of pollinating and non-pollinating fig wasps, and explain spatiotemporal variation observed in fig wasp abundances. In this study we investigate the combined effect of multiple biotic and abiotic factors on the reproductive dynamics of one pollinator wasp and eight non-pollinating fig wasp species interacting with *Ficus petiolaris*, a rock-strangler fig tree endemic to Mexico. Because our main objective was to investigate the effects of these factors on the mutualism through pollinator production and pollen dispersal, we also investigated their relationship to the overall production of pollinators versus non-pollinating fig wasps. This research topic is important because fig trees are often identified as keystone species in tropical ecosystems (Terborgh, 1986; Lambert and Marshall, 1991; Shanahan et al., 2001; Serio-Silva et al., 2002; Harrison, 2005; Kissling et al., 2007), and biotic and abiotic environments are rapidly changing due to anthropogenic led global climate change and natural habitat fragmentation.

Here we quantify fig wasp production at the level of individual syconia sampled from trees growing at nine geographic locations spanning the range of *F. petiolaris* in Baja California. For each wasp species, and for pollinators versus non-pollinating fig wasps, we examine the association between offspring production and biotic factors associated with syconia characteristics (size, foundresses, nematode infection), fig tree size and reproductive phenology (synchronous/asynchronous), and the local abundance of syconia on the landscape. As weather can be very different from one sampling periods or location to another (Figure 7; Supplemental Figure 2), we also consider land surface temperature and precipitation as abiotic factors. We applied generalized linear mixed models to examine the linear relationship between fig wasp abundance and these factors, with the goal of identifying factors resulting in higher costs or benefits for the mutualists (Figure 1). We use this approach to test the following hypotheses relating to wasp production: 1) increasing foundress number will increase pollinating wasp production, favoring pollinators over non-pollinating fig wasps; 2) asynchronous flowering favors non-pollinating fig wasps over pollinating fig wasp production because non-pollinating fig wasps are better able to cycle within natal trees; 3) fig tree crown volume and reproductive effort will increase the floral volatile signal attracting fig wasps, resulting in higher visitation and offspring production of all individual species; 4) syconia abundance in the landscape will differentially affect fig wasp species, exhibiting a positive correlation with the per-syconium production of poor dispersers and negative correlation with better dispersers; 5) near-surface temperatures exceeding 33-38°C should negatively impact all fig wasp species but favor pollinators over non-pollinating fig wasps due to the shorter exposure of pollinators to extreme temperature once inside syconia; 6) rainfall events should negatively impact all fig wasp species but have a reduced impact on pollinators over non-pollinating fig wasps because more of their

adult life-cycle is spent protected inside syconia. As species abundances and community dynamics often vary in space and time, we studied the *F. petiolaris* system across sites separated by up to 741 km and over four collecting trips spanning two wet/dry seasons.

We were also interested in the relationship between *F. petiolaris*' reproductive phenology and its abiotic environment. We expected that high temperature and dry climate might constraint *F. petiolaris*' flowering, and that, conversely, rainfall and cooler temperatures will allow emergence of large, synchronous crops. Thus, we conducted supplemental analyses to test the following hypothesis: 7) lower temperature and higher precipitation will favor reproduction in *F. petiolaris* and are associated with higher reproductive effort and synchronous flowering.

2. Materials and methods

2.1. The biological system

Ficus petiolaris consists of three subspecies (Piedra-Malagón et al., 2011) and is endemic to Mexico, distributed primarily in the western part of the country from Baja California and Sonora in the north to Oaxaca in the south (Serrato et al., 2004; Piedra-Malagón et al,. 2011, Ibarra-Manríquez et al., 2012). Compared to other New World strangler figs (subgenus Urostigma, section Americana), *F. petiolaris* is unusual as a rock-strangling tree growing on rock patches, often leading to small but relatively dense populations. In the desert of Baja California, it is the only native fig and one of only a few tree species producing leaves and fruits year-round, including during the summer when temperatures frequently exceed 38°C. *F. petiolaris* is used by local human populations for therapeutic practices and the mature figs can be prepared as marmalade for consumption. These large distinctive evergreen trees are often used as shrines for spiritual and religious purposes (J.D. Nason, personal observation).

Ficus petiolaris is pollinated by one specific, undescribed fig wasp species of the genus *Pegoscapus* Cameron (Agaonidae, Gates & Nason, 2012; Duthie & Nason, 2016; Piedra-Malagón et al., 2018) and is also associated with nine undescribed species of non-pollinating fig wasps: 4 species of *Idarnes* Walker (family Agaonidae, two of the species group *carme* and two of the species group *flavicolis*), two species of *Heterandrium* Mayr (family Pteromalidae), one species of *Ficicola* Heydon (Pteromalidae, misidentified in Duthie et al., 2015 and Duthie & Nason, 2016, corrected by J.Y. Rasplus, pers. comm.) and its potential parasitoid, a species of *Physothorax* (Torymidae), and finally, one species of *Sycophila* Walker (Eurytomidae), which is only rarely represented in this system. As only one species of *I. flavicolis* occurs in Baja California, we will consider only *I. flavicolis* sp. 1. The biologies of non-pollinating fig wasps is not known with certainty but previous studies have highlighted the feeding strategies of wasps within the genera cited above. *Idarnes* represents common non-pollinating fig wasps in the New World (Bouček, 1993). First considered gallers competing for ovules with pollinators (West and Herre, 1994), species in this genus were then observed reaching receptive syconia at different times suggesting different feeding strategies (Elias et al., 2008). More recently, Elias et al. (2012) reported two *Idarnes* feeding strategies on *F. citrifolia*, a species of the *I. flavicolis* group identified as a galler and a species of the *carme* group observed without ambiguity to be a cleptoparasite. Observations on *F. petiolaris* suggest that *I. flavicolis* sp. 1 oviposits eggs in *F. petiolaris*' syconia around the same time as pollinators arrive on receptive figs, while *I. carme* sp. 2 often arrives later and *I. carme* sp. 1 only after pollination (J.D. Nason, pers. obs.) which tends to support observations of Elias et al. (2012) on *F. citrifolia*. The biology of *Heterandrium* is not well known, as these wasps are usually less common and considered either gallers or parasitoids (West et al., 1996). Information on *Ficicola* is sparse but a species has recently been

characterized as a gall inducer because the wasps oviposit into pre-receptive syconia (A phase) before pollination (Conchou et al., 2014). The same study concluded that *Physothorax* is likely a parasitoid of *Ficicola*, as suggested by Bouček (1993). Wasps from the genus *Sycophila* also have a poorly known biology and they are assumed to be wasps with broad host range (Boucek et al., 1981). Li et al., however, used molecular methods to argue in favor of cryptic species and host specificity within the genus (2010) and Darwell et al. (2019) argued that *Sycophila* are parasitoids.

Ficus syconia also contain nematodes that are carried by infected pollinators (Martin et al., 1973), and *F. petiolaris* pollinators and non-pollinating fig wasps are frequently infected by a species of *Parasitodiplogaster* nematodes (Van Goor et al., 2018). High levels of infection (>10 per host) have significant impacts on the lifespan and dispersal of *Pegoscapus* sp. (Van Goor et al., 2018), as well as several non-pollinating fig wasps (Van Goor, unpublished data). Pre-dispersal predation by *Omiodes stigmosalis* caterpillars on developing syconia is also observed in *F. petiolaris* (Piatscheck et al., 2018) but is not considered in this study as syconia predation results in the death of all wasp offspring, regardless of species.

2.2. Site distribution and sampling

Ficus petiolaris trees were geo-referenced at nine sites along a latitudinal gradient spanning 741 km of the Baja California peninsula (Piatscheck et al., 2018, Van Goor et al., 2018, Table 1, Supplemental Figure 1). Fig tree study sites were visited at four time points (November-December 2012, May-July 2013, November-December 2013, and May-July 2014) to ensure adequate sample sizes of wasp producing figs in both fall (October-December) and spring (May-July) seasons. During site visits, we collected mature syconia (wasp releasing, D phase), in

which we measured syconia-related variables (foundress count, syconia volume and nematode infection), counted emerging wasps, and measured fig tree reproduction variables (reproductive asynchrony and reproductive effort). Some sites in some sampling periods had no flowering fig trees, which resulted in missing data for wasps counts, syconia and fig tree reproduction variables. Details of flowering trees observed and D phase syconia collection can be found in both Table 1 and Supplemental Table 1.

2.3. Wasp collection and identification

Mature figs were sampled from each site to determine the numbers of pollinating and non-pollinating fig wasps produced per fig. An objective was set of more than 100 wasp releasing syconia (D phase) from several fig trees per sites. During the four visitations of the nine sites, we collected a total of 2367 syconia that had no exit hole yet. They were spit in half then quickly placed in a vial. Because some wasps had not yet emerged from their gall, we placed the vials containing syconia under a light source overnight. The wasps were then collected from each vial and syconium and stored in 95% ethanol and were kept at temperature between 1 - 6 °C before transport to Iowa State University for further processing. Once in the laboratory, all pollinating and non-pollinating fig wasps per syconium were identified to species and sex.

2.4. Syconia-level predictor variables

After collecting the emerging wasps, for each D-phase syconum we measured the length, width and height to estimate syconium volume. We also counted the number of dead pollinating wasp foundresses, and recorded the presence or absence of dauer-stage infective

Parasitodiplogaster nematodes. These juvenile nematodes perform nictation behavior to increase contact with wasp hosts and are easily recognizable when present within D-phase syconia.

2.5. Tree-level predictor variables

Patterns of reproductive phenology and syconia production have been described for *F. petiolaris* in Piatscheck et al. (2018). Following this publication, for tree-level predictor variables we estimated reproductive asynchrony (an index ranging from 1 to 7, where 1 = synchronous flowering with all syconia in the same phase and 7 = asynchronous flowering with syconia evenly distributed across all stages) and reproductive effort (≤ 5%, 5 – 25%, 25 – 50%, 50 – 75%, 75 – 95%, or ≥ 95% of maximum capacity, which would be branches covered with syconia).

2.6. Neighborhood-level predictor variables

We used the approach of Nottebrock et al. (2016) to create a neighborhood-level measure of *F. petiolaris* reproductive activity, which serves as an explanatory variable we call the *syconium landscape*. Here the reproductive activity of individual trees was estimated as the product of their reproductive effort (see Section 2.5) and crown volume (m^3). For each focal plant we then summarized the reproductive activity of neighboring plants as a decreasing function of their distance from the focal plant within a user-defined distance radius. Specifically, the distances d_{ij} between the focal plant i and the neighboring plants j are calculated, and the syconium landscape c_i for plant i is estimated as the sum of the products of the reproductive activity of plant j times the negative exponent of the squared distance d_{ij}^2 over a spatial scaling parameter σ^2. Parameter σ^2 controls the strength of the negative exponent and the rate at which

the contributions to c_i of plants j decrease with distance. As we have no *a priori* knowledge of the effect of syconium landscape, we analyzed our data with respect to c_i calculated for radii of 50 m, 100 m, 250 m and 500 m, and σ set at half or one-fifth of the value of the radius (allowing for greater and lesser effects of more distant trees, respectively). Although the syconium landscape was measured at the time wasp-releasing D-phase syconia were collected from focal tree i, we use it as a proxy for the amount of syconia produced by neighboring trees at the time wasps were laying eggs into tree i's syconia. We expected that syconium landscape would covaries with local tree density (the greater the host tree density the shorter the distances d_{ij} between the focal plant i and the neighboring plants j).

2.7. Abiotic predictor variables

Successful dispersal of fig wasps to and oviposition in receptive syconia may be influenced by local climatic conditions, which in turn may impact the production of pollinating and non-pollinating fig wasps produced per fig. Temperature and precipitation at specific sites can be quite different across visit times (illustration with weather variation during field trips with the city of La Paz in Supplemental Figure 2). To examine the effect of climatic variables on fig wasp production, we therefore estimated maximum daytime temperature and precipitation at the time sampled syconia were pollinated and parasitized by non-pollinating fig wasps. We acquired daily, site-specific maximum daytime land surface temperature data from MODIS (from MOD11A1 data set, Wan et al., 2015), which are estimates derived from satellite imagery. We similarly acquired precipitation data from CHIRPS (Funk et al., 2015). These data were acquired as daily averages for the years 2012, 2013 and 2014. Syconia development from pollination and oviposition to wasp emergence takes approximately five weeks in the summer (J. Nason,

unpublished data) to an estimated eight weeks in the winter. Since it is unknown precisely when the syconia we sampled began their development, we averaged the local temperature and precipitation data over the period of six to ten weeks (one month total) prior to syconia collection.

2.8. Analysis of temporal and geographic variation of biotic and abiotic reproductive variables

We first used R (R Core Team, 2018) to obtain summary statistics (proportions, means and standard deviations) of all response and predictor variables. Predictor variables are *syconia volume*, *nematode presence*, *foundress count*, *tree volume*, *land surface temperature* and *precipitation*. Response variables are wasp count per species (eight variables), and some variables were used both as predictor and response: *within-tree asynchrony* and *reproductive effort*. Then, we investigated potential temporally and geographically significant variation of response and predictor variables with R. Investigation of deviance from the global mean across sites and seasons for all but one predictor variable were performed with factorial ANOVAs in R. Nematode infection variation across sites and seasons was also assessed, however, nematode infection data are presence/absence (i.e., binary) and the variable was analyzed with a G-test of independence with the package DescTools (Signorell et al., 2019). We analyzed fig wasps species composition produced per syconium across sites and seasons with a factorial MANOVA with the package *stats* in R, and we performed principal component analyses (PCA) to visualize the variation in wasp communities at the levels of syconia and trees with the package *vegan* in R (Oksanen et al., 2018). Figures were created with *ggplot2* (Wickham, 2016).

2. 9. Modeling the ecological correlates of *F. petiolaris* reproductive variables

To test potential significant associations between *F. petiolaris* reproductive variables and the abiotic environment, we modeled the relationship between flowering *within-tree asynchrony* as a response variable and *land surface temperature* and precipitation as predictor variables using linear mixed models, conducted using the function lmer in the package *lme4* (Bates et al., 2015). Linear mixed models were also used to model the relationship between *reproductive effort* an the predictor variables *land surface temperature* and *precipitation*. To investigate an the presence of any latitudinal trends of *F. petiolaris* reproductive characteristics, we also added population *latitude* in the models. *Collecting trips* (i.e., fall 2012, spring 2013, fall 2013 and spring 2014) and *trees nested within sites* were treated in the model as random variables. To equalize the contributions of predictor variables in the linear mixed models, all were standardized to reduce their range from 0 to 1 ($x = (x - \min(x) / (\max(x) - \min(x))$).

2.10. Modeling the ecological correlates of foundresses counts

Mixed models were used in R to test the association between pollinator *foundress count* and predictor variables *tree volume*, *reproductive effort*, *within-tree asynchrony* and *neighboring syconium landscape*. However, as the response variable is a count of pollinator per fig, we use appropriate generalized linear mixed models assuming Poisson distributed errors, conducted using the function *glmer* in the package *lme4* (Bates et al., 2015). The random variables incorporated in the mixed models were *collecting trips* and *trees nested within sites*. We standardized the predictor variables as in section 2.8.

2.11. Modeling the ecological correlates of pollinator and non-pollinating fig wasps production

We modeled the relationship between species wasp offspring counts per syconium and multiple biotic and abiotic predictor variables using generalized linear mixed models assuming Poisson distributed errors. For each response variable corresponding to one of the nine species of fig wasps, the wasp count was modeled with the counts of the seven other species in the syconium (i.e., each species count representing one predictor variable), *syconium volume* and *foundresses count*, within-tree *asynchrony* indices, *reproductive effort*, tree *volume*, *neighboring syconium landscape*, *temperature* and *precipitation*, as the 16 predictor variables in these models. Random effects and standardization of the predictor variables were as in sections 2.8 and 2.9.

To study the effect of fixed and random variables on the cost benefits involved in the mutualism (pollinator reproduction and its pollen disperser function), we used total pollinator counts and total non-pollinating fig wasp counts per syconium as log-odds for a logistic regression with *syconium volume* and *foundresses count*, *asynchrony*, *reproductive effort*, *tree volume*, *neighboring syconium landscape*, *temperature* and *precipitation* as predictors. The models used were generalized linear mixed models with a binomial error distribution. Random effects and standardization of the predictor variables were the same as in sections 2.8 and 2.9. A significant positive association with a fixed variable indicates increasing number of pollinators relative to non-pollinating fig wasps correlated with increasing values of this variable, thus benefiting the mutualism. Conversely, a significant negative association with fixed variable indicates decreasing number of pollinators relative to non-pollinating fig wasps correlated with increasing values of this variable, signifying a cost for the mutualism.

3. Results

3.1. The fig wasp community associated with *F. petiolaris*

We collected 2367 mature, D phase *F. petiolaris* syconia across sites and collecting trips, with the average syconium producing 91.23 adult fig wasps (median of 75, range from 2 - 547). The proportions of pollinating and non-pollinating fig wasps across sites and collecting trip are represented in Figure 2. Average numbers of the different wasp species per syconium are summarized by site and collecting trip in Figure 3. Although the *Pegoscapus* pollinator was the most common species (41.12% of all wasps), non-pollinating fig wasps in aggregate comprised the majority of wasps produced per syconium (58.88%; Figure 2). *Idarnes flavicolis* sp. 1 was the most abundant non-pollinating fig wasp species (17.69%, females only) followed by *Idarnes carme* sp. 1 and sp. 2 (6.15% and 10.33%, respectively, females only). Including *Idarnes* males (which were not identified to species), the three *Idarnes* together represented 50.17% of all *F. petiolaris* wasps. Interestingly, one or more *Idarnes* species was found in 95.48% of all syconia, whereas *Pegoscapus* was found in 76.47% of syconia. *Heterandrium sp.1, Heterandrium sp.2*, *Ficicola*, *Physothorax* and *Sycophila* were less common (3.88%, 2.46%, 0.83%, 1.46% and 0.08%, respectively) but still common in the system, found in 49.22%, 44.32%, 25.10%, 31.35% and 3.59% of syconia, respectively. Thus, all but *Sycophila* were found in more than one fourth of mature syconia. A detailed summary of wasp species abundances per syconium is presented in Supplemental Table 1.

The 2367 collected syconia averaged 1.32 *Pegoscapus* foundresses (Figure 4B), but often had several foundresses and in one case more than 20 foundresses. 14.79% of the syconia with *Pegoscapus* foundresses (typically one) produced no offspring. The lack of pollinator offspring could be due to premature collection of syconia, though offspring of other wasp species were

observed. 7.56% of syconia had no foundresses and, overall, 23.53% syconia produced no pollinator offspring. These D phase syconia without *Pegoscapus sp.* all produced non-pollinating fig wasps, primarily *Idarnes*, but in three instances where only a few *Heteranrium* sp. 1 or sp. 2 were present, in two instances where a few *Physothorax* were present., and in a single syconium with a few *Ficicola* sp. or *Sycophila* individuals. Although most of the syconia without pollinator offspring contained at least two species of *Idarnes*, we also collected 165 syconia with only *Idarnes flavicolis* sp. 1 females (94 without *Pegoscapus* foundresses), 11 syconia with only *Idarnes carme* sp. 1 females (3 syconia without *Pegoscapus* foundresses), and 17 syconia with only *Idarnes carme* sp. 2 females (4 syconia without *Pegoscapus* foundresses). Illustrative of the high frequency of non-pollinating fig wasp, we observed only 3.67% syconia in which non-pollinating fig wasps were absent.

The results of the factorial MANOVA analyzing fig wasp species composition indicate significant differences between sites and seasons, with a significant interaction term (Table 2). This highlights extensive spatial and temporal variation in wasp communities. PCAs of wasp species composition confirm this aspect of wasp community variation at the syconia and tree levels (Figure 5). Broadly overlapping ellipsoids in the PCA show that wasp community composition does not predictably cluster by site or collecting season. Results of best fit generalized linear mixed models indicate numerous significant linear associations between fig wasp species production per syconium and biotic and abiotic predictor variables (Table 3). Interestingly *Pegoscapus* abundance is negatively associated with *Idarnes flavicolis* sp. 1 abundance while being positively associated with *Idarnes carme* sp. 1 and sp. 2 abundances. The associations of *Pegoscapus* with other wasp species vary (positive to negative) and are all significant. Associations of *Pegoscapus* with *Heterandrium* sp. 1 and sp. 2 are of opposite sign

(positive and negative respectively) and, in general, *Pegoscapus* is negatively associated with larger wasps (*Heterandrium* sp. 1, *Ficicola.* and *Physothorax*). Among non-pollinating fig wasps, *Idarnes flavicolis* sp. 1 is significantly negatively associated with *Idarnes carme* sp. 1 and sp. 2, while the two *Idarnes carme* species are positively correlated. *Ficicola* and *Physothorax*, which are biologically associated as host and parasitoid, respectively, are positively associated in the generalized linear mixed models. Although *Sycophila* was rare, it is found in syconia with higher abundances of *Idarnes carme* sp. 1, *Heterandrium* sp. 1, and *Ficicola.*

3.2. Temporal and geographic variation of biotic and abiotic variables

ANOVAs and G-tests of independence of biotic and abiotic variables (Table 2) all showed significant deviation from the grand mean across *F. petiolaris* sites, seasons, and their interaction, indicating high spatial and temporal variation in these variables. The volume of mature syconia averaged 1960 $mm^{3,}$ but as illustrated in Figure 4A, differed substantially within sites across collecting trips; indeed sometimes more than two-fold (e.g., sites 172, 113, 95, and 201). Some of these syconia were collected from the same trees across trips, but most of the time from different trees. As documented previously by Van Goor et al. (2018), *Parasitodiplogaster* nematode infestation of *F. petiolaris* syconia was 34.9% (Figure 4C) and varied significantly across collection trips and site (Supplemental Table 2, $G = 287.61$, X-squared $df = 25$, $p\text{-value} < 2.2e\text{-}16$). We did not observe nematodes at site 179 in spring 2014, otherwise the syconia infestation levels ranged from 7.90% to 77.10%.

The heights of *F. petiolaris* trees ranged from less than 1 m for very small non-flowering individuals to more than 25 m, with an average crown volume of 347 m^3 (Supplemental Table 3). Northern populations tended to have smaller trees (2.83 m average height at site 158 in the

extreme north *versus* 10.08 m at site 70 in the extreme south), but large trees were also be found at latitudinally intermediate sites (e.g., site 113 with average height 10.75m), especially if found near a seasonal watercourse. Phenological measurements were also highly variable within and across sites (Figure 6, Supplemental Table 2). Reproductive trees were usually found bearing only a few syconia (a small fraction of potential reproductive output), often in multiple developmental phases (89.52% of all reproductive trees had asynchronous syconia development), though very large and highly synchronous crops were also observed. We found 18 instances in which *F. petiolaris* trees bore syconia in all phases of development. Characteristic of other variables, the syconium landscape produced by neighboring trees was also highly variable (Supplemental Table 2).

Temperature and rainfall in Baja California are typical of the Sonoran Desert climate experiencing seasonal storms. Figure 7 illustrates variation in these climatic variables for a representative location in southern Baja over a ten-year period that includes our four collecting trips. Fall trips tended to be preceded by heavier rainfall, which were stronger in 2012 than in 2013, while spring trips were preceded by drier and warmer weather. Climate summaries for six to ten weeks prior to our *F. petiolaris* sampling (see section 2.6) varied considerably between sites (Supplemental Table 2), with more variation in daily mean land surface temperature in the northern sites than in southern sites (data not shown). Although we expected northern sites to be dryer, sites 158 and 112 in the north experienced the most rainfall, and contrary to our expectation, higher land surface temperature were often observed in the spring in southern locations. This latter trend is likely influenced by our collecting populations from north to south, with southern sites visited later in the spring when the weather was generally warmer and drier. The result is that climate variables exhibit clear latitudinal trends (Supplemental Table 2),

highlighting the importance of taking sampling period and current local weather conditions into consideration.

3.3. Modeling the ecological correlates of *F. petiolaris* reproductive variables

Analysis of tree-level *reproductive effort* (measured relative to maximum potential output) indicated a significant positive association with *tree volume* and *precipitation*, and a significant negative association with *temperature* Table 4). *Within-tree asynchrony* was similarly associated with *temperature* and *precipitation*, while the effect of *tree volume* was not significant. Reproductive effort was greater in larger trees and during cooler and wetter weather, while asynchrony was greater under cooler and wetter conditions. Interestingly, *reproductive effort* did not follow a latitudinal trend whereas asynchrony was less common at lower latitude.

3.4. Modeling the ecological correlates of foundresses counts

Generalized linear mixed models revealed no significant association of *foundress counts* with *within-tree asynchrony*, *reproductive effort*, *tree volume*, *neighboring syconium landscape* or *precipitation*. However we notice a positive association with *temperature* (estimate = 0.28, $P = 0.03$, AIC = 3446.6).

3.5. Modeling the ecological correlates of pollinator and non-pollinating fig wasps production

Generalized linear mixed models were used to determine how offspring production per syconia by individual fig wasp species is influenced by other *F. petiolaris* wasp species and multiple environmental variables. We consider the results of these generalized linear mixed models in three parts, examining first individual wasp species production in response to the

production of the eight other wasp species, second to other biotic variables (*within-tree asynchrony*, *reproductive effort* and *volume*, and *neighboring syconium landscape*), and third to abiotic variables (*temperature* and *precipitation*). Although nine species of wasps were investigated, we are primarily interested in how the production of *Pegoscapus* pollinator offspring per syconium covaries with the abundances of each of the eight species of non-pollinating fig wasp. *Pegoscapus* is a galler that competes with members of the *Idarnes flavicollis* group for ovules. We therefore expected the abundance of *Pegoscapus* offspring to have a significant negative association with *Idarnes flavicolis* sp. 1 abundance, which our generalized linear mixed model analysis revealed to be the case (Table 3). *Pegoscapus* is subject to cleptoparasitism by members of the *Idarnes carme* group, and as a host its abundance should covary positively and significantly with *Idarnes carme* sp.1 and sp. 2, which it does. *Pegoscapus* similarly covaries positively with *Sycophila*, a potential parasitoid. *Pegoscapus* abundance is significantly negatively associated with the abundances of *Ficicola* and *Physothorax*, which are the largest non-pollinating fig wasps (although these large wasps have mixed effects on the other non-pollinators). The biology of *Heterandrium* is not well known but the association of *Pegoscapus* with *Heterandrium* sp. 1 and sp. 2 is significantly negative and positive, respectively, suggesting the two congeners may have rather different life histories.

Generalized linear mixed models in which the individual non-pollinating fig wasps are response variables and *Pegoscapus* a predictor variable reveal patterns of covariation between species that are very similar to that summarized in the preceding paragraph (Table 3). Among non-pollinating fig wasp associations with each other, *Idarnes flavicolis* sp. 1 was significantly negatively associated with both *Idarnes carme* sp.1 and sp. 2, which covaried positively with each other (Table 3). Associations of these three *Idarnes* with the remaining non-pollinating fig

wasps were variable, for example, with *Idarnes flavicolis* sp. 1 and *Idarnes carme* sp. 2 having significant positive associations with *Heterandrium* sp. 1 and sp. 2, while *Idarnes carme* sp. 2 had significant negative associations with these two species. Conversely, despite their differences in association with *Pegoscapus*, the two *Heterandrium* species responded similarly to the abundances of the three *Idarnes* species. *Ficicola* and *Physothorax* have a host-parasite relationship, respectively, and as with *Pegoscapus* and its cleptoparasites and potential parasitoid, they too exhibit a significant positive association. Because *Physothorax* develops in galls initiated by *Ficicola*, we expected these two species to have similar associations with the other non-pollinating fig wasps, but their effects were quite variable. The putative parasitoid *Sycophila* had a positive association with other similar sized wasps (*Pegoscapus*, *Idarnes*, and *Heterandrium*), though significantly so only for *Idarnes flavicolis* sp. 1. Unexpectedly, it also has a strong significant positive statistical association with the much larger *Ficicola*, though not its *Physothorax* parasitoid.

Host-related biotic variables were significantly associated with *Pegoscapus* wasps. *Syconia volume*, *foundress number*, *reproductive effort, neighboring syconium landscape* were positively associated with *Pegoscapus* while negative associations are observed between *Pegoscapus* and reproductive variables *within-tree asynchrony* and *tree volume* (Table 3). Similar trends were observed in *Idarnes flavicolis* which was, however, positively associated with *tree volume*. It was also negatively associated with *foundress count.* Interestingly, the regression slope of the association between *Idarnes flavicolis* and *neighboring syconia landscape* is large. *Idarnes carme* species show very different trends than observed in *Pegoscapus* and *Idarnes flavicolis*. *Idarnes carme* sp. 1 shows a positive association with foundress number whereas *Idarnes carme* sp. 2 shows a similar trend which is not significant, but is positively

associated to larger syconia. Both *Heterandrium* species are positively associated with *within-tree asynchrony* and negatively associated to *foundress count*. Both *Ficicola* and its putative parasitoid *Physothorax* are in higher abundance in larger syconia, however *Ficicola* is positively associated with *foundress count* whether *Physothorax* is negatively associated with this variable. *Sycophila* is not associated with any biotic variables related to the host fig biology.

The abiotic environment also showed some interesting results on individual species counts. Most notably, *Pegoscapus sp.* wasps being positively associated with day *land surface temperature* and *precipitation*. In *Idarnes flavicolis*, however, both these variables are negatively associated with their counts. These two species, which are the most abundant, show here very dissimilar responses to the climate. A positive association with *Idarnes carme* sp. 1 and temperature can be observed, as well as in *Ficicola*. *Heterandrium* sp. 1, however is negatively associated with *precipitation*. Details are found in Table 3.

Results of the logistic regression indicate which environmental factors favor pollinators over non-pollinating fig wasps (Table 3). We found that *syconia volume* and *foundress count* result in syconia producing larger number of pollinators relative to non-pollinating fig wasps, that *within-tree asynchrony* favors non-pollinating fig wasps, and fig *tree volume*, *reproductive effort* and *neighboring syconium landscape* had no effect (marginal significance for the latter). Interestingly, higher *land surface temperature* and greater *precipitation* are positively associated with more of pollinator offspring, resulting in lower non-pollinating fig wasps, when lower temperature and precipitation were recorded.

4. Discussion

4.1. *Ficus petiolaris* and associated insects: a highly variable system

Fig wasp communities are usually dominated numerically by the pollinator, but for a few Neotropical *Ficus* species the combined abundance of non-pollinating fig wasps can reach or exceed that of the pollinator species. *Ficus petiolaris* supports one of these unusual communities, with the frequency of non-pollinating fig wasps produced per syconium being both variable in space and time, and typically very high (Figure 2; Supplementary Table 1). Indeed, at site 158 in fall 2012, 97.18% of the 3083 wasps reared from mature syconia were non-pollinating fig wasps. To illustrate the variability observed across seasons, collections at this same site during two subsequent field trips (spring 2013 and spring 2014) contained 71.57% non-pollinating fig wasps (5627 wasps collected) and 50.96% non-pollinating fig wasps (1197 wasps collected), respectively. Site 158 is at the northern limit of the range of *F. petiolaris* in Baja California, but other sites also had high and variable proportions of non-pollinating fig wasps (Figure 2; Supplementary Table 1). The lowest proportion of non-pollinating fig wasp observed for a single collecting trip was at site 113 (central Baja) in fall 2013 (20.10%), but during the preceding and following collecting trips (fall 2012 and spring 2014) we collected 83.98% and 92.38% non-pollinating fig wasps, respectively. Southern sites too have large and variable proportions of non-pollinating fig wasps, for example ranging from 38.49% to 80.47% across collecting trips at site 96. Even though it was not one of our main interests, we also documented nematode infection rates across syconia in *F. petiolaris.* Our results again suggest spatial and temporal variation in nematode infection and that this should be taken into consideration while studying nematode infestation in *Ficus.*

Initially, we expected smaller sized *F. petiolaris* populations to be subject to greater exploitation by non-pollinating fig wasps. Since it is our sense that northern populations are smaller and more isolated from each other than southern ones, we expected an increase in the proportion of non-pollinating fig wasp per syconium with latitude. Evidence from other frost-sensitive Sonoran Desert plants indicates that northern Baja California populations are younger, have smaller effective sizes, and were established post-Pleistocene via range expansion from ancestral refugial populations located in central and southern Baja (Nason et al., 2002; Clark-Tapia & Molina-Freaner, 2003; but see Garrick et al., 2009). Fig population size may impact the relative abundances of pollinating and non-pollinating fig wasps because smaller populations of figs are thought to have greater within-tree reproductive asynchrony as an adaptation to promote the persistence of pollinators by enabling them to emerge from and enter figs on the same host tree (Janzen, 1979). Genetic analyses by Gates & Nason (2012), however, found no evidence of selfing and inbreeding in *F. petiolaris*, as would be expected from pollinators cycling within host trees, presumably because the abortion of selfed syconia exerts strong selection on pollinators to disperse to other trees. They conclude that the primary benefit of within-crown asynchrony is that it favors successful dispersal of pollinators and outcross reproduction of figs by increasing the frequency and temporal overlap of wasp releasing and receptive syconia on neighboring trees. Importantly, they note too that asynchrony has the potential cost to the mutualism of enabling non-pollinating fig wasps to cycle and increase in abundance within the crowns of individual *F. petiolaris* trees. Despite lacking information on population sizes, our analysis investigating the association between asynchrony and latitude confirm these thoughts. Further analyses investigating *F. petiolaris* population sizes by estimating effective population size based on genetic data for example, would bring insights to this association.

4.2. *Ficus petiolaris*' reproductive characteristics

The reproductive phenology of Baja California populations of *F. petiolaris* has been previously described by Gates & Nason (2012) and Piatscheck et al. (2018), who also reported substantial variation in reproductive activity across sites and seasons, as well as in within-tree reproductive asynchrony. Identifying mechanistic linkages between fig reproductive biology and local climatic variables has proven to be a challenge. In gynodioecious species, production of B and D phase syconia is reportedly influenced by seasonality (Kuaraksa et al., 2012, Chiu et al., 2017), but seems to vary greatly among species (Bain et al., 2014). In monoecious species, however, studies of reproductive phenology have shown mixed results. In the old world, *Ficus microcarpa*, for example, shows, in some cases, no association between crop production and rainfall (Corlett, 1984, similar results with *F. benjamina*), lower reproduction in cold and dry weather (Lin et al., 2008), or higher reproduction in warmer weather in other cases (Yang et al., 2013). Similarly, *F. subpisocarpa* and *F. caulocarpa* were observed producing figs year-round, with 30% of trees bearing syconia, but no associations with local climate were found (Chiang et al., 2018). In a study of new world monoecious figs, five Panamanian species tended to produce crops year-round but with a peak in crop initiation during the hotter dry season (Windsor et al., 1989). In *F. petiolaris*, we found that reproductive phenology was significantly associated with both the precipitation and temperature that occurred six to 10 weeks prior (Table 4). Specifically, our results indicate that larger and more synchronous crops are favored under cooler and more humid climatic conditions. Within-tree synchrony in *F. petiolaris* could potentially be triggered by rainfall events, which are more frequent during cooler weather, in contrast to long period of drought usually observed during warmer weather. The same is true for the initiation of large crops. In contrast, low temperatures to disfavor syconia production in old world monoecious fig

species (Lin et al., 2008). However, the authors add that low syconia production happen in particularly cold and dry months.

Variation in syconia volume was partially explained by the number of foundresses that entered syconia and by higher temperature and more rainfall. Larger syconia are known to produce more galled flowers and a more pollinator offspring (Cook & Power 1996, Sun & Wang, 2019). Because syconia contain a greater proportion of pollinators in warmer and wetter environments, it is not surprising to find these variables correlating with larger syconia. A similar pattern was observed in *F. racemosa* in southwestern China, where D-phase syconia (unlike B-phase syconia) were found to have a larger diameter in dry warm seasons as opposed to rainy and foggy cool seasons (Zhang et al., 2006). However, Zhang et al., focused on the phenology and growth of syconia and not on the associated fig wasp community, so it is not possible to directly compare the potential mechanisms of syconium size variation between *F. petiolaris* and *F. racemosa*.

4.3. Interpretation of non-pollinating fig wasp biologies from wasp counts and ecological correlates

While it has generally been assumed that members of *Idarnes flavicolis* are gallers (West et al., 1994, 1996; Pereira et al., 2007; Elias et al., 2008), a study by Elias et al., (2012) provide the only direct assessment of their oviposition behavior to date. They demonstrated that an *Idarnes flavicolis* species associated with *F. citrifolia* in Brazil oviposits into and galls flowers in a manner similar to that of the *Pegoscapus* pollinators, and that pollination, and the presence of pollinator larvae and seeds, are not required for their successful development. In *F. petiolaris*, we frequently observed syconia that were successfully parasitized by *Idarnes flavicolis* sp. 1 in the absence of developing pollinators (6.25% of 2367 syconia sampled). Some of these syconia also

contained *Heterandrium, Ficicola, Physothorax* and/or *Sycophila*, but these non-pollinators are uncommon and instances where *Idarnes flavicolis* sp. 1 occurred alone were relatively frequent. This indicates that, as with the *Idarnes flavicolis* species studied by Elias et al. (2012), *Idarnes flavicolis* sp. 1 can develop in *F. petiolaris* syconia without relying on other wasp species, which is consistent with their being gallers. Because *Idarnes flavicolis* sp. 1 and the pollinator develop in the same inner ranks of flowers (J. Nason, unpublished data) and therefore likely directly compete for fig resources, we expected a negative correlation between the abundances of these two wasp species in syconia. Consistent with this expectation, our generalized linear mixed models indicate significant negative associations between *Pegoscapus* and *Idarnes flavicolis* sp. 1 abundance (Table 3). *Heterandrium* sp. 1 and sp. 2 are found in much lower abundances than *Idarnes* species, but both are positively associated with *Idarnes flavicolis*, which may indicate parasitism. Both species have been observed without *Idarnes flavicolis* wasp in syconia. *Heterandrium* sp. 2 is also positively associated with the polliantors but have in many instances been found without them. Interestingly, both *Heterandrium* species are positively associated with each other, a trend that we expect from host-parasitoid associations.

Parasitoid abundances are often positively correlated with host density, as they tend to aggregate where hosts are abundant (Waage, 1983; Roland & Taylor, 1997; Umbanhowar et al., 2003), but their interactions with hosts are also variable across habitat patches and environments (Vinson, 1976; Pacala et al., 1990; Meisner et al., 2014). Previous investigations in *Ficus* have shown positive correlations (Peng et al., 2005), no correlation (Suleman et al., 2013) or negative correlations (Kerdelhué et al., 2000, Raja et al., 2015) between host and parasitoids wasp offspring abundances within syconia. In Raja et al., the negative correlation between host, the pollinator *Kradibia tentacularis*, and parasitoid, *Sycoscapter* sp., was observed only in

experiments controlling for host foundress number, and not syconia from semi-natural populations where foundress number, and thus the number of potential hosts, was unaccounted for. Nonetheless, significant correlations between fig wasp host and parasitoid have been observed in natural populations where host foundress number was unknown (Peng et al., 2005; Kerdelhué et al., 2000). Where hosts are common and utilize most or all of the available resources within a syconium then parasitoids (and cleptoparasites) will necessarily be negatively associated with host abundance. However, two conditions could contribute to positive host-parasitoid correlations in abundance. For one, where hosts are uncommon and found in only a subset of syconia, then parasitoids will necessarily be found in these same syconia. For another, where these hosts typically utilize only a subset of resources available within a syconia, then a greater abundance of hosts may be associated with a greater abundance of parasitoids.

In the *F. petiolaris* fig wasp community, we have identified one host-parasitoid and two host-cleptoparasite associations. *Ficicola* and its *Physothorax* parasitoid are both uncommon and, consistent with our predictions, they exhibit strong positive associations in abundance within syconia (Table 3). The *Pegoscapus* pollinator is believed subject to cleptoparasitism by the two *Idarnes carme* species. Although *Pegoscapus* is relatively abundant, accounting for 41% of all wasps, it averages 37.5 offspring per syconia, which is well less than the more than 250 pollinators capable of being produced per syconium. For its relationship with both *Idarnes carme* sp. 1 and sp. 2, we also expected – and observed – positive correlations in abundance (Table 3). These similar positive associations with *Pegoscapus* can be attributed to similar feeding behavior in by these *Idarnes carme* cleptoparasites. However, for both *Idarnes carme* species, we occasionally found offspring developing in syconia that contained neither *Pegoscapus* foundresses nor developing offspring. Even if these instances are infrequent relative to the total

number of syconia sampled, they demonstrate that *Idarnes carme* wasps are not obligately associated with *Pegoscapus* and may rely on other other gallers (e.g., *Idarnes flavicollis* sp. 1) as hosts.

Sycophila is reported to be a parasitoid of a diversity of insect hosts, including wasps associated with *Ficus* (Boucek et al., 1981). Compared to other wasps in the *F. petiolaris* community, *Sycophila* has strong positive associations with *Idarnes flavicollis* sp. 1 and *Ficicola* (Table 3), suggesting that they may serve as hosts for *Sycophila*. Interestingly, *Idarnes flavicollis* sp. 1 and *Ficicola* are not so strongly associated with each other. Also, *Sycophila* and *Idarnes flavicollis* sp. 1 have similar body sizes while *Ficicola* is much larger. This suggests that perhaps multiple *Sycophila* may develop within a single *Ficicola* host.

4.4. Environmental correlates with wasp counts

Host-related biotic factors affected wasp counts in various ways. Fig trees with higher reproduction were associated with higher counts of the two most common species in this system (*Pegoscapus* and *Idarnes flavicolis*), suggesting that fig trees with more figs and, possibly releasing higher amounts of attractant volatiles, may be more attractive to these specialized wasps than trees with lower reproduction. Although the ecological function of these volatiles has been well characterized (Hossaert-McKey 1994, Borges et al., 2008), no one, to our knowledge, has investigated the relationship between fig plant-level reproductive effort and floral volatile production. Floral display size is known to influence rate of pollinator visitation (Eckhart, 1991; Brody & Mitchell, 1997). Like floral display, a large chemical display by highly reproductive fig trees could potentially attract more wasps. Our results suggest, but do not demonstrate, such a trend. Further research on fig plant-level reproductive effort and floral volatiles production could

resolve this issue. Similarly, *Pegoscapus* is positively associated with neighboring syconium landscape. This follows our expectation that the neighboring syconium landscape should be correlated with fig tree connectivity. A previous study demonstrated this association between *Pegoscapus* and *F. petiolaris* (Duthie & Nason, 2016). It was surprising, however, to observe a negative correlation with *Idarnes flavicolis.* This is counter-intuitive because, as a short disperser, we expected to observe it more in tree patches with higher connectivity (Duthie et al., 2015). It suggests however that neighboring flowering trees can influence the rate of visitation of fig wasps, may be via the release of volatile attractants. Clarification will require further research on the effect of landscape-level fig tree floral scent effect on fig wasps. Tree volume was significantly associated with wasp count in three species: *Pegoscapus*, *Idarnes flavicolis* and *Idarnes carme* sp.1. A tree's size could act as a complement for fig wasp attractiveness with fig chemical volatiles. However, the negative association between tree volume and *Pegoscapus* and *Idarnes carme* was unexpected and remains difficult to interpret.

Within-tree asynchrony is common in *F. petiolaris,* as suggested by our observation, yet selfing is absent (Gates & Nason, 2012). Although the cycling of pollinators within their natal tree can be rejected, within-tree asynchrony effect on non-pollinating fig wasp reproductive dynamics was not known. Previous authors suggested that within-tree asynchrony would affect the dynamics of non-pollinating fig wasps (Cook & Power, 1996, Gates & Nason, 2012) but evidence was lacking. A recent study showed mixed effects on pollinator/parasite production (Krishnan et al., 2014). Here we show that increased within-tree asynchrony is significantly associated with higher abundance of three species of non-pollinating fig wasp: *Idarnes carme* sp. 1, both species of *Heterandrium* (and marginally significant with *Idarnes carme* sp. 2). Conversely the association between within-tree asynchrony and pollinator count is negative,

which could be attributed to non-pollinators laying egg into figs first because of their proximity to receptive syconia, leading to fewer flowers available for the pollinator to lay eggs in. Overall, within-tree asynchrony has a strong negative impact on the reproduction of pollinator relative to non-pollinating fig wasps. This finding confirms previously stated hypotheses which anticipated higher rate of parasitism in within-tree asynchronous trees.

The change in the abiotic environment, especially temperature, has rightfully raised concerns for fig wasps (Jevanandam et al., 2013; Sutton et al., 2018). The fig wasps, particularly pollinators, are known to have a short life (Kjellberg et al., 1988), and recently, experimental studies have shown their sensitivity to temperature above 33°C, which are particularly lethal above 38-40°C. However, our results show that, from a species community perspective, temperature can potentially have a positive effect on pollinating fig wasps. The most common parasite *Idarnes flavicolis* is negatively associated with temperature. Conversely, *Pegoscapus* is positively associated with land surface temperature, possibly attributable an ability for the pollinators to lay more eggs inside syconia. when its most common parasite is reduced. Indeed, we propose that higher temperatures do not benefit the pollinator when outside the syconia, but benefit once inside the syconium where they are protected from the heat, while their parasites are still exposed to it. Surprisingly we did not see other negative associations between temperature and non-pollinating fig wasp counts. Instead, some are positive as in *Idarnes carme* sp. 1 and *Heterandrium* sp. 2. It is intriguing how *Pegoscapus* and *Idarnes* sp. 1 have similar responses to environmental variation, perhaps suggesting a tighter interaction than described in section 4.2. Overall, increased temperature benefits pollinator production. Similarly, increased rainfall should negatively affect non-pollinating fig wasp leading to an advantage for the pollinator. Our results support this hypothesis as several non-pollinating fig wasp species are negatively associated with

rainfall (*Idarnes flavicolis* and *Heterandrium* sp. 1) and corresponding to a positive association with the pollinator. Overall, increased rainfall largely benefits the pollinator.

Future climate is expected to become hotter, dryer and more extreme in Baja California (Cavazos & Arriaga-Raminez, 2012; Vaghefi et al., 2017). Ongoing anthropogenic global climate change is predicted to affect species interactions and community structure in various ways (Tylianakis et al., 2008; Walther, 2010), and mutualisms in particular (Dunn et al., 2009; Yang & Rudolf 2010; Kiers et al., 2010). Here we present an unexpected potential benefit of climate change to the fig – fig wasp mutualism by benefiting pollinator production. Future research will use models of climate change scenarios predict the fig wasp community structure into the future.

5. Conclusion

Variation in the environment is important in species interaction dynamics (Thomson, 1981; Travis, 1996; Chamberlain et al., 2014; Friberg et al., 2019). Here we present work on fig wasp community reproductive dynamics in an extensively characterized environment including both host-related biotic factors and important aspects of the abiotic environment. The high variability observed in the *F. petiolaris* system highlights the need to collect extensive spatial and temporal data to characterize the reproductive dynamics of figs and their associated wasp communities. Our results certainly would have been misleading if we had focused on only a single study location (as has been the case in many studies of figs and their wasps) or based our inferences on samples obtained from a single collecting trip. Further, incorporating sites and seasons as random variables in generalized linear mixed models of wasp species production per syconium improved model fitting scores and our ability to identify significant biotic and abiotic

correlates of wasp production (Table 3). Other plant-insect interaction systems likewise exhibit high geographic and temporal variation (Olesen & Jordano, 2002; Manzaneda et al., 2007; Dupont et al., 2009) which emphasize that observations made from one specific geographic location and period are likely to highlight context dependent dynamics, and thus potentially misrepresent broader-scale dynamics and processes. To our knowledge, no other studies have examined the extensive spatial and temporal variation of a fig wasp community. We argue that observational fig wasp community studies should integrate spatial and temporal perspective into their design, and we advise caution when interpreting the dynamics of species communities in a single environmental context.

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Table 1. *Ficus petiolaris* site information listed from north to south, including numbers of mapped trees and incidence of reproductive trees observed by season of collecting trip.

Sites	Latitude, longitude	Georeferenced trees	Reproductive trees (any syconia phase)			
			Fall 2012	Summer 2013	Fall 2013	Summer 2014
158	29.2628391; -114.021134	103	12	24	25	25
172	28.2677527; -113.1095672	70	24	17	22	20
112	27.56043406; -113.0671864	75	9	19	31	42
113	27.0995915; -112.4968451	76	33	18	25	35
95	26.3598798; -111.8040866	101	22	12	30	31
179	25.91341244; -111.3514215	38	16	0	15	22
201	25.37795264; -111.3125629	42	8	4	8	19
96	24.0459034; -110.1344447	337	35	26	36	49
70	23.73747426; -109.8275927	105	24	43	43	44

Table 2. Analysis of variance of season and site effects on biotic variables used as predictor variables in analyses of *F. petiolaris* wasp abundances(see Table 3).

Response variables	Season			Site			Season*Site		
	Df	*F*	Pr(>*F*)	Df	*F*	Pr(>*F*)	Df	*F*	Pr(>*F*)
Syconia volume	3	48.95	***	8	66.37	***	21	55.14	***
Foundress counts	3	29.20	***	8	9.16	***	21	14.81	***
Reproduction	3	17.18	***	8	4.37	***	20	3.04	***
Asynchrony	3	0.03	*	8	2.76E-13	***	21	8.58E-10	***
Tree volume	3	0		8	44.84	***	24	0	
Syconium landscape	3	20.35	***	8	49.12	***	24	15.73	NA

Table 3. Regression parameter estimates from best fit Poisson regression models of counts of individual *F. petiolaris* wasp species produced per fig, as well as logistic regression models of counts of pollinating relative to non-pollinating fig wasps. In addition to the indicated fixed-effect predictor variables, random effects include collecting trip, sites, and fig trees nested within sites. Significance: *** = <0.001, ** = <0.01, * = <0.05, . = <0.1 (marginally significant); Poll = pollinators; NPFW = non-pollinating fig wasps; LST = land surface temperature; NA = not applicable.

Predictor variables	*Pegoscapus* sp.	*Idarnes flavicolis* sp. 1	*Idarnes carme* sp.1	*Idarnes carme* sp. 2	*Heterandrium* sp. 1	*Heterandrium* sp. 2	*Ficicola* sp.	*Physothorax* sp.	*Sycophila* sp.	Poll./ NPFW
Pegoscapus sp.	NA	**-0.85** ***	**1.89** ***	**1.07** ***	**-1.75** ***	**2.55** ***	-0.16	**-2.22** ***	1.14	NA
Idarnes f. sp. 1	**-0.96** ***	NA	**-0.78** ***	**-0.86** ***	**1.55** ***	**3.15** ***	0.16	0.04	**4.58** **	NA
Idarnes c. sp. 1	**0.95** ***	**-0.22** **	NA	**0.72** ***	**-0.57** **	**-0.22 .**	**1.22** ***	**-0.62** *	0.53	NA
Idarnes c. sp. 2	**0.56** ***	**-0.37** ***	**0.84** ***	NA	0.53 ***	**0.77** ***	0.19	**0.50** *	1.64	NA
Heterandrium sp. 1	**-1.31** ***	**0.87** ***	**-0.66** ***	**0.53** ***	NA	**1.05** ***	0.18	0.49	1.93	NA
Heterandrium sp. 2	**1.98** ***	**2.60** ***	**-0.40** **	**0.97** ***	**1.47** ***	NA	0.29	**-2.13** ***	0.66	NA
Ficicola sp.	**-0.61** ***	-0.18	**1.62** ***	-0.31	-0.16	0.06	NA	**4.16** ***	**7.81** ***	NA
Physothorax sp.	**-0.92** ***	0.10	**-0.69** ***	0.35	0.24	**-1.65** ***	**2.22** ***	NA	-1.69	NA
Sycophila sp.	**1.90** ***	**1.20** **	0.43	**1.99** **	0.59	-1.21	**10.62** ***	**-4.34** *	NA	NA
Syconium volume	**2.69** ***	**1.58** ***	0.02	**0.39** *	**1.46** ***	**-1.48** ***	**2.38** ***	**0.88** *	**-5.13** *	**2.39** ***
Foundress count	**1.60** ***	**-0.40** ***	**0.61** ***	**0.22 .**	**-1.96** ***	**-0.60** *	**0.89** *	**-0.77** *	-1.25	**2.60** ***
Tree volume	**-3.23** **	**8.63** ***	**-3.77** **	-1.16	-0.92	0.20	0.13	**-2.56 .**	-3.02	-1.69
Reproduction	**0.37** ***	**2.46** ***	-0.03	0.67 *	0.35	-0.42	0.41	-0.68	-1.63	-0.02
Asynchrony	**-2.24** ***	**-3.95** ***	**2.08** ***	**0.72 .**	**1.18** **	**2.46** ***	-0.21	0.68	-0.12	**-1.16** ***
Syconium landscape	**2.56** ***	**-13.22** ***	**5.50** ***	**2.02 .**	0.08	0.30	0.08	**2.72.**	4.56	**1.02 .**
LST	**0.60** ***	**-3.60** ***	**0.88** *	0.52	-0.84	**2.25** ***	-0.68	0.15	1.73	**1.70** ***
Precipitation	**2.37** ***	**-1.89** ***	0.04	0.37	**-1.50** **	-0.76	-0.67	-0.73	3.79 *	**3.23** ***
AIC	**35065.7**	**20585.1**	**12717.5**	**15356.8**	**9311**	**6425**	**3750.5**	**4002.6**	**544.1**	**29277.1**

Table 4. Regression coefficient estimates from best fit Gaussian regression models of *F. petiolaris*' reproductive variables.In addition to the indicated fixed-effect predictor variables, random effects include collecting trip, sites, and fig trees nested within sites. To reflect land surface temperature and Precipitation effects at the time of wasp dispersal and oviposition, these predictor variables were estimated 8 weeks prior to syconia collection. Significance: *** = <0.001, ** = <0.01, * = <0.05, . = <0.1 (marginally significant); LST = land surface temperature; NA = not applicable.

	Predictor variables			
Response variables	Latitude	Tree volume	LST	Precipitation
Reproduction	-0.00	0.63**	-0.24 ***	0.09 **
Asynchrony	-0.04*	-0.19	-0.22 ***	-0.23 ***

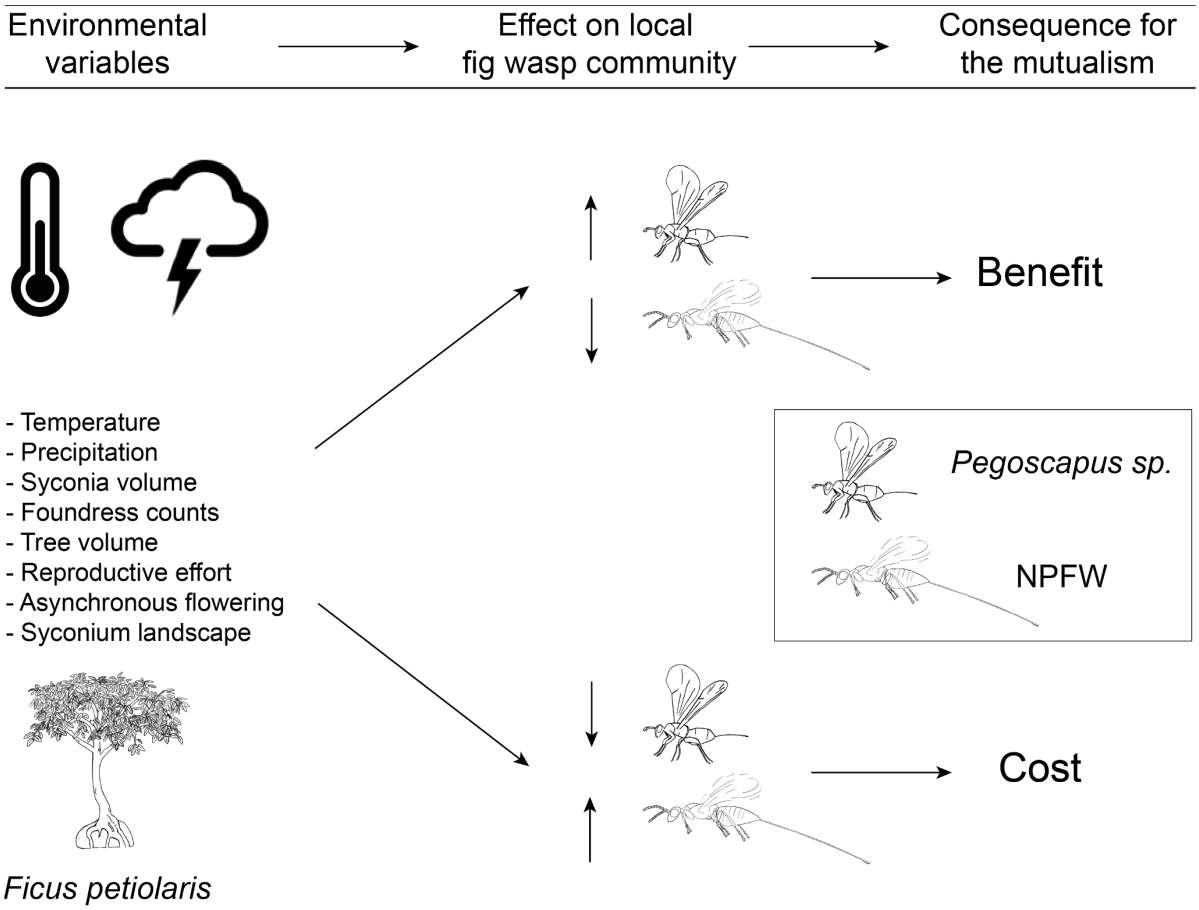

Figure 1. Conceptual diagram summarizing the tested variables in this study and their combined potential effect on the fig wasp community and consequences for the fig-fig wasp mutualism. Environmental variables combine two abiotic variables and six biotic variables associated with syconia and *F. petiolaris*' biology. The combined effect of these variable could locally increase the proportion of pollinators relative to non-pollinating fig wasps which would result in a benefit for both symbionts, or conversely increase the proportion of non-pollinating fig wasps which would negatively impact the mutualism. NPFW = non-pollinating fig wasps.

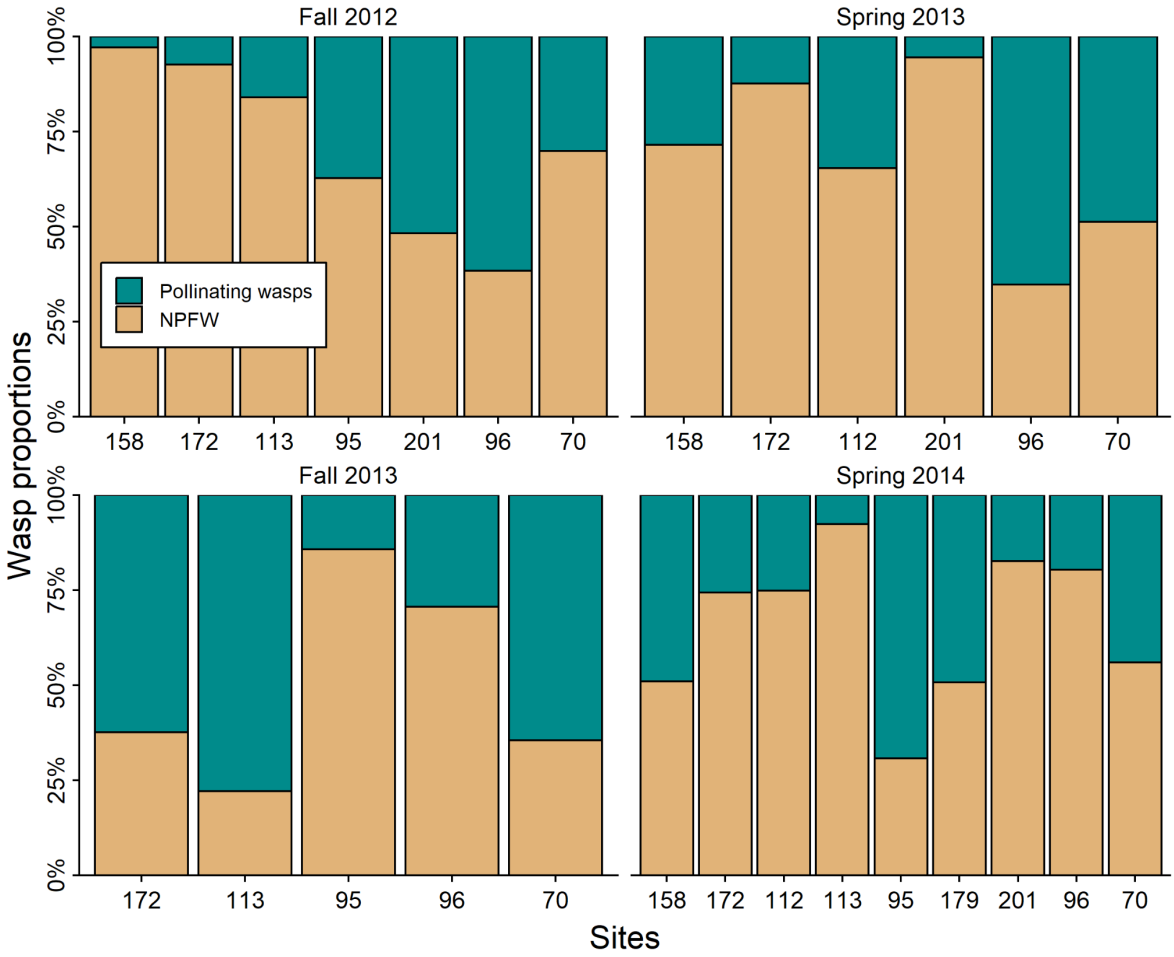

Figure 2. Proportion of pollinating wasps and non-pollinating fig wasps at specific sites during the four collecting seasons. Missing wasp proportion for a few sites represent failed attempts to collect mature wasp-releasing syconia. NPFW = non-pollinating fig wasps.

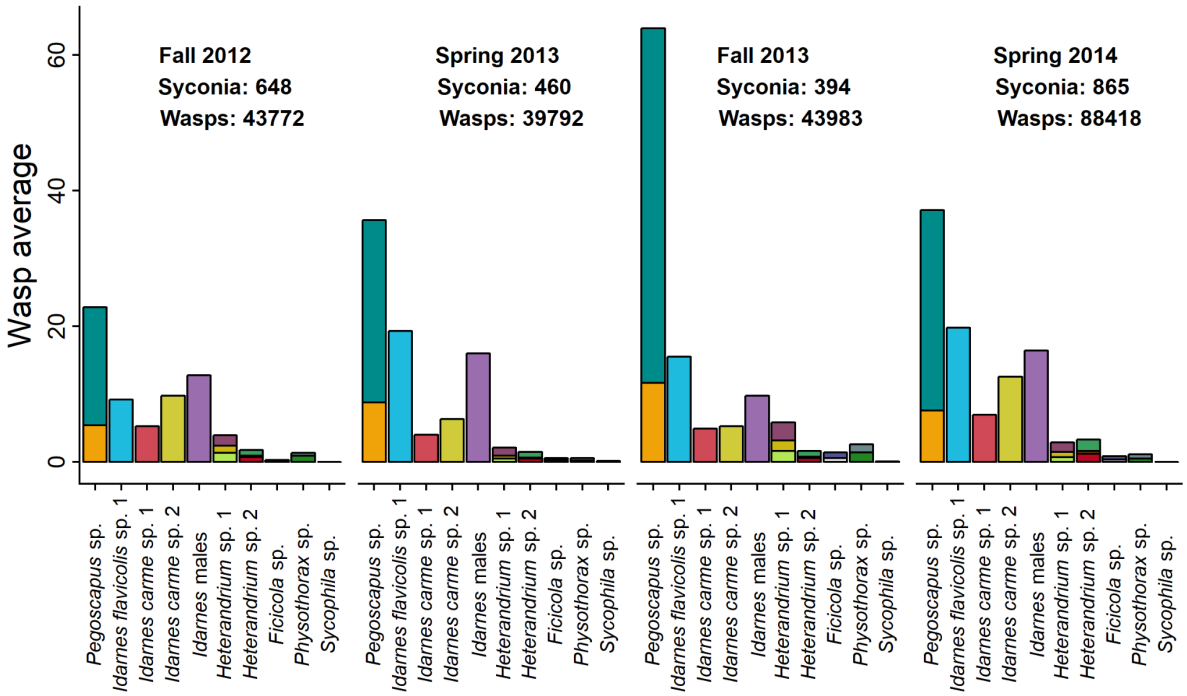

Figure 3. Wasp count averages per syconium across sites and seasons. Divisions within bars separate females (upper section) and males (section(s) below).

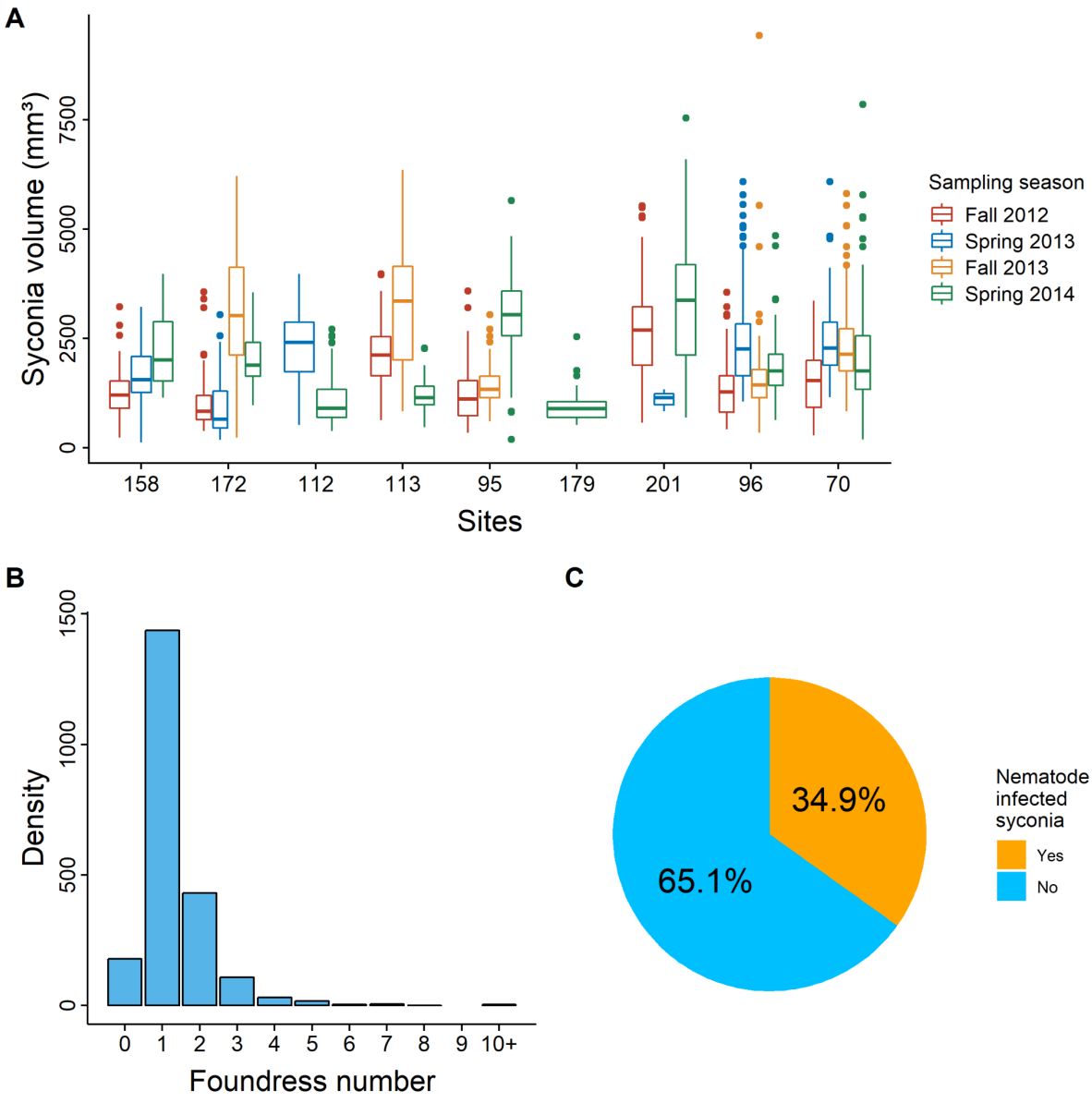

Figure 4. Variables associated with syconia produced by *F. petiolaris.* A) Syconia volume boxplots represented across sites and seasons from most northern site on the Baja peninsula (158) at the left and most southern site (70) at the right, B) histogram representing number of foundresses collected per syconium and C) proportion of the infested syconia by *Parasitodiplogaster sp*. nematodes in *F. petiolaris*.

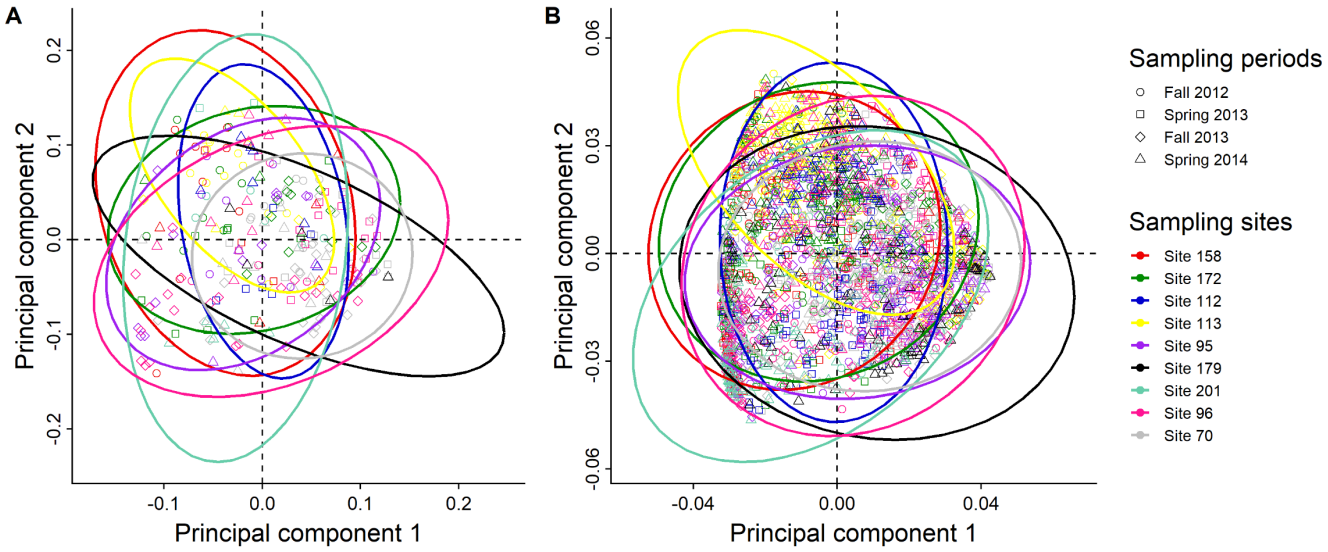

Figure 5. Axes describing the most the variability from a principal component analysis of the wasp community compositions in syconia represented by collecting sites and seasons. A) points are wasp community means per trees, B) points are wasp community counts per syconia. Colors represent syconia from the nine sites visited and shapes represented the sampling trips. Ellipses are drawn for data per sites, ellipses for data grouped by seasons are also overlapping but not represented here for better visibility.

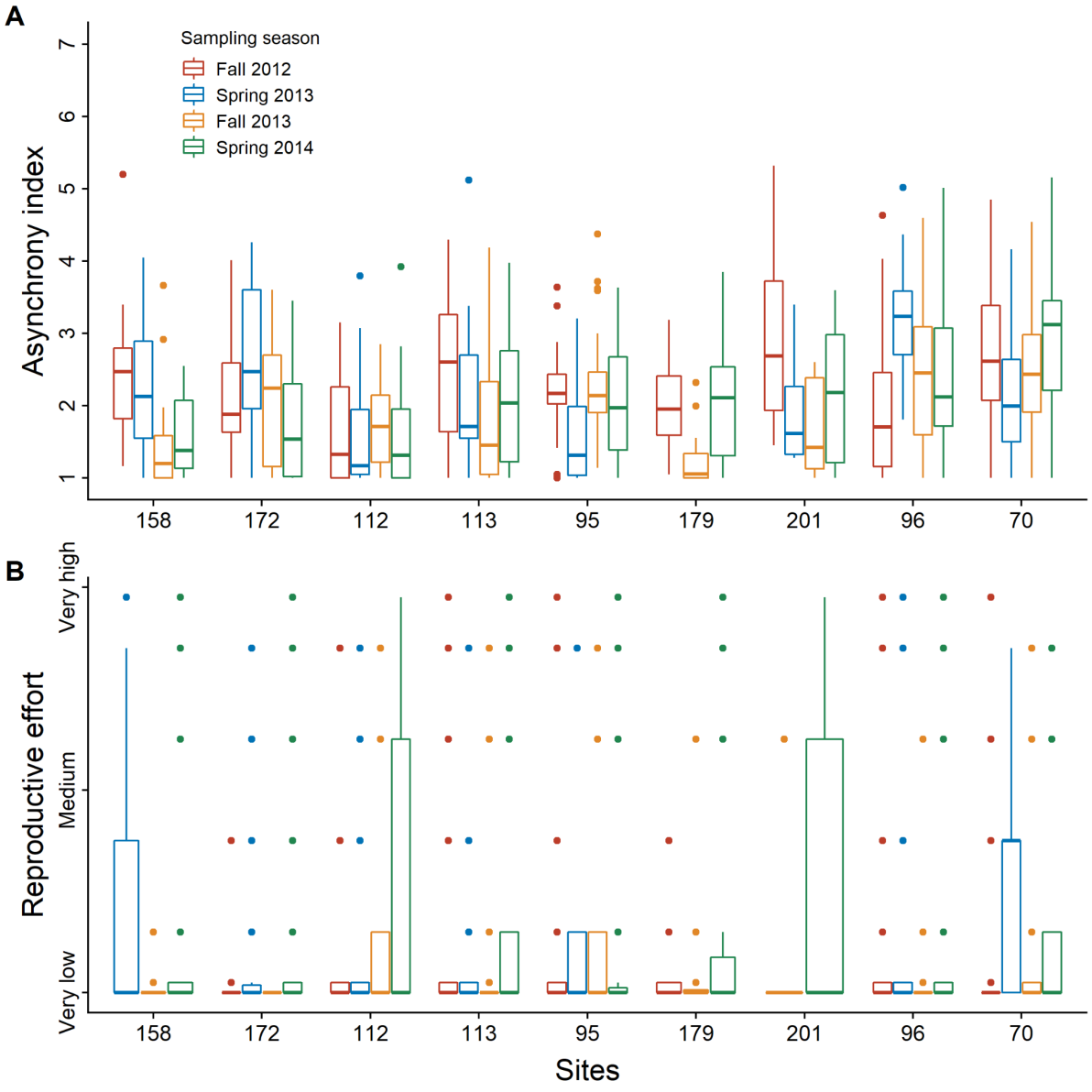

Figure 6. Boxplots representing the phenological variables of *F. petiolaris.* A) Within-tree reproductive asynchrony variation across sites and seasons and B) reproductive effort variation across sites and seasons. Sites are arranged from most northern site in the peninsula (158) at the left and most southern site (70) at the right.

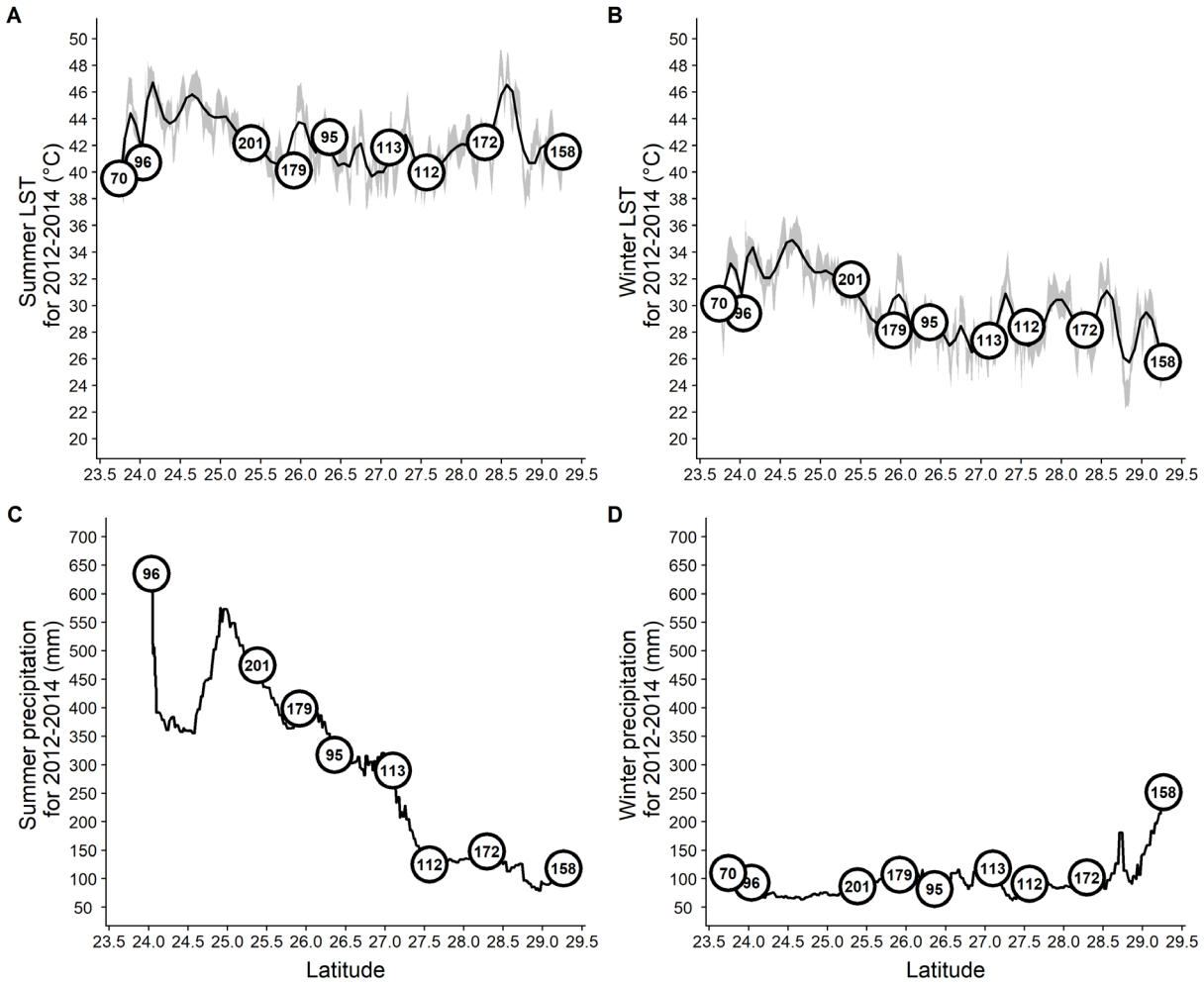

Figure 7. Mean annual land surface temperature (A and B) and precipitation (C and D) for three years by latitude from CHIRPS and MODIS (MOD11A1) respectively, represented by season. Summer based on data from 21 March to 22 September, and winter based on data from 23 September to 20 March. The data were obtained for coordinates along transects passing through the study sites (highlighted in the figures), and avoiding the sea.

CHAPTER 4. ECOLOGICAL FACTORS ASSOCIATED WITH PRE-DISPERSAL PREDATION OF FIG SEEDS AND WASPS BY FIG-SPECIALIST LEPIDOPTERAN LARVAE

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Abstract

In brood pollination mutualisms, predation of developing fruit can have large negative repercussions for both plant and pollinator population dynamics. The Sonoran Desert rock fig *Ficus petiolaris* and its highly-coevolved wasp pollinator are subject to frequent attack by lepidopteran larvae that consume fig fruit and the developing seeds and larval pollinators they contain. We used generalized linear mixed models to investigate how the phenology, quantity, and spatial distribution of fig fruits is associated with variation in lepidopteran damage intensity on individual trees at nine geographic locations spanning a 741 km latitudinal transect along Mexico's Baja California Peninsula. We found lepidopteran damage to be strongly positively associated with more synchronous fig crops and larger trees, and only weakly associated with local host tree density. These results imply that fruit production that is asynchronous within trees and spread out over time, as observed in several fig species, benefits female and male components of fitness (pollen disperser and seed production, respectively) by reducing pre-dispersal predation by frugivores.

1. Introduction

The majority of flowering plants rely on animals for pollination services while pollinators, in turn, obtain valuable plant resources (Ollerton et al., 2011). The success of this mutualistic interaction depends on numerous ecological factors, as the plant-pollinator partners may be differentially affected by both biotic and abiotic environments. For example, numerous studies have investigated climate variation effects on plant phenology as well as temporal mismatches between plants and pollinators that their negatively impacts on mutualistic outcomes (Harrison, 2000; Wall et al., 2003; Memmott et al., 2007; Burkle et al., 2013; Rafferty et al., 2013; Rafferty et al., 2015). Theoretical and empirical studies of optimal foraging also indicate plant density and per-plant production of floral resources to be positively associated with pollinator attraction, floral visitation and, ultimately, seed production (Platt et al., 1974; Silander, 1978; Klinkhamer & de Jong, 1990; Kunin, 1993, 1997), while decreases in both the density and spatial continuity of natural plant populations negatively impact pollinator visitation rates and plant reproductive success (Nason & Hamrick, 1997; Tscharntke & Brandl, 2004; Aguilar et al., 2006; Potts et al., 2010). Such studies are of growing importance since the global-scale increase in average temperature is causing a temporal shift towards earlier flowering (Fitter & Fitter, 2002; Walther et al., 2002; Cleland et al., 2007; Franks et al., 2007; Cook et al., 2012) and human-mediated landscape fragmentation is already advanced and increasing (Vitousek et al., 1997; Achard et al., 2002; Hansen et al., 2013), which together are driving concerns about both temporal and spatial mismatches in plant-pollinator interactions.

In addition to mutualistic interactions with insect pollinators, flowering plants are ubiquitously subject to antagonistic interactions from a diverse assemblage of phytophagous insects (including herbivores, florivores, seed predators, and frugivores), which may also be

subject to environmentally-driven spatial and temporal mismatches with host plants (Thompson and Gilbert, 2014). A number of studies have investigated host plant density and frequency effects on insect herbivory (Root, 1973; Bach, 1980, 1988; Fagan et al., 2005) and phenological mismatches in plant-antagonist interactions (Augspurger, 1981; Brody, 1997; Wisser et al., 2001; Singer and Parmesan, 2010; Yang and Rudolf, 2010; Liu et al., 2011; Rafferty et al., 2013). Few studies, however, have jointly investigated spatial and phenological effects on plant-pollinator-herbivore interactions (but see Parsche et al., 2011). Concerning phenology, the theoretical study by Fabina et al. (2010) indicates that changes in phenological overlap in these interactions can alter the relative population densities and persistence of all three symbionts in ways not predicted solely from their pair-wise interactions. This suggests that temporal and spatial variation in climate may simultaneously affect the phenological overlap/mismatch of pollinators, phytophagous insects, and a shared host plant, and, consequently, the outcome of their species interactions.

Researchers considering the consequences of changes in flowering phenology have focused on the distribution of flowering time among individuals within and among populations. Virtually unstudied is the variance in flowering phenology *within* individual plants and, in turn, its effects on antagonistic interactions with phytophagous insects. While more asynchronous flowering may potentially ameliorate environmentally-driven phenological mismatches with pollinators, it may similarly benefit antagonists and, thus, have unappreciated costs for mutualism. Flowering phenology, resource availability (flower or fruit production), and plant density might thus be factors potentially affecting the plant-phytophagous insect dynamic. We expect that 1) within-plant asynchronous flowering will extend the phenological overlap between plant resources and insect antagonists leading to greater costs to the plant, 2) larger crops of

resources will experience greater damage than smaller ones, and 3) fruit damage will increase with local host plant density. We tested these predictions by investigating interactions between a native fig and an associated pre-dispersal fruit-feeding insect in Baja California, Mexico.

Figs (*Ficus*, Moraceae) are diverse (750+ species) and widely distributed across tropical and subtropical environments worldwide (Berg, 1989). *Ficus* is typically one of the most speciose genera in tropical forests, with members functioning as keystone species because their aseasonal fruit production provides food vital to the survival of many vertebrate frugivores, especially during seasons when fruiting activity of other plant species is low (Terborgh, 1986; Lambert and Marshall, 1991; Shanahan et al., 2001; Serio-Silva et al., 2002; Harrison, 2005; Kissling et al., 2007). Contributing to reproductive isolation where they co-occur, each fig species typically has a species-specific relationship with a single pollinating chalcidoid wasp species (family Agaonidae; superfamily Chalcidoidea) that develops within galled fig seeds before mating within host inflorescences (syconia, commonly called fig fruits). The reproductive phenology of figs is unusual in that flowering is generally highly synchronized within trees and yet highly aseasonal at the population level. Moreover, the production of male and female flowers is separated by several weeks so that to interbreed trees must be phenologically out of phase with each other. In some species, however, trees exhibit substantial within-crown flowering asynchrony and may simultaneously bear syconia at various stages of development (Smith & Bronstein, 1996; Gates & Nason, 2012). This asynchrony may be of substantial benefit to the reproductive success of figs and pollinators by increasing overlap in flowering times and, hence, opportunities for pollinator and pollen transfer between trees (Gates & Nason, 2012). It is expected to be particularly beneficial where host populations are small and spatially isolated (Bronstein et al., 1990; Anstett et al., 1995; Anstett et al., 1997; Gates & Nason, 2012).

In addition to providing an excellent model system for studying obligate mutualism, figs are exploited by a diversity of non-pollinating insects. The best known of these are non-pollinating fig wasps (multiple chalcidoid families) that reproduce within fig syconia (West et al., 1996; Weiblen, 2002; Borges, 2015). These non-pollinating wasps exploit the mutualism in various ways and are generally associated with a decrease in pollinator production, and to a lesser extent seed production (West and Herre, 1994; Kerdelhue et al., 2000). Less well studied are the myriad of other animals that are antagonist of the fig-pollinator mutualism, including moths. In the Sonoran Desert rock fig, *Ficus petiolaris*, an undescribed moth species (family Crambidae) lays eggs on or under the cuticle of the fig syconium, with later instar caterpillars boring into syconia and consuming developing seeds and wasps. Although few cases of fig boring lepidopteran caterpillars have been reported in the literature (New World: Janzen, 1979; Bronstein, 1988; Jandér, 2015; Old World: Sugiura and Yamazagi, 2004), we observed lepidopteran damage to the syconia of *F. petiolaris* to not only be common but to sometimes be drastically high, destroying all syconia with their developing seeds and pollinators within a tree. *Ficus petiolaris* thus provides a useful and interesting biological system to study the effects of flowering phenology, crop size and plant density on levels of attack by a phytophagous insect that has significant consequences on host fitness

For this study we first obtained samples of the moth antagonist of *F. petiolaris* from nine sites spanning its range in Baja California, Mexico, and employed mitochondrial sequences and phylogenetic tools to confirm its species delimitation and determine its taxonomic placement within the Crambidae. We then used generalized linear mixed models to test whether within- and among-site variation in fig damage caused by the moth larvae are related to measures of asynchronous flowering, crop size and/or local fig tree density. These models enabled us to test

our *a priori* hypotheses, namely that each of the three predictor variables will be positively associated with levels of caterpillar damage and fruit loss.

2. Materials and methods

2.1. The biological system

Ficus petiolaris is an evergreen rock-strangling fig tree occurring within Mexico from Oaxaca in the south to the Sonoran Desert environments of Sonora and Baja California in the north. Because *F. petiolaris* grows on rocks, cliffs or canyon walls, it forms small natural population patches on rocky landscapes. Tree size varies greatly and can reach 20+ meters in height (Finn Piatscheck, pers. obs.). As a member of subgenus *Urostigma* (section *Americana*), this species is monoecious with syconia containing both female and male flowers. In contrast to most other *Americana* species, however, syconia production in *F. petiolaris* is often asynchronous within individual trees (Smith & Bronstein, 1996; Gates & Nason, 2012). *Ficus petiolaris* is pollinated by a single species of chalcid wasp (*Pegoscapus sp*., Agaonidae), with this mutualism being exploited by several obligately-associated insects, including eight species of non-pollinating chalcidoid wasps and one species of crambid moth. Extensive surveys of *F. petiolaris* trees and surrounding vegetation have located larvae of this moth only on fig trees (J. Nason, pers. obs.), consistent with it being a fig specialist. Moth larva typically bore into syconia near the peduncle and an individual late-instar caterpillar consumes the interior of several syconia - including seeds and wasp larvae - during its development. Indeed, after consuming the inside of one syconium, a caterpillar will move to another syconium (often the nearest one), chew its way inside, and use latex produced by the fig mixed with other material to then close the entry hole (F. Piatscheck, pers. obs.). In addition, a caterpillar will often feed within a pair of

adjacent syconia connected via a tunnel it constructs from latex material, an unusual behavior further suggesting fig specialization. Oviposition by female moths and movement of larvae between syconia is exclusively nocturnal, and fifth instar caterpillars drop by night on silken threads from *F. petiolaris* trees to pupate in the soil. Any fig damaged by a caterpillar will abort with the loss of all remaining developing seeds and pollinators. The total damage to a tree can be substantial as we have in some cases observed fruit crops of *F. petiolaris* in which 100% of syconia were consumed by the caterpillars.

2.2. Site distribution and sampling

Field data were collected from nine sites distributed along a 741 km transect on Mexico's Baja California peninsula (Figure 1, Table 1). At each site, we established a census population of geo-referenced *F. petiolaris* trees. Field data, including measures of reproductive activity and lepidopteran damage per tree, were obtained during three extended field trips: during the dry season in spring (May-June) 2013 and 2014, and during the wet season in fall (November-December) 2013. Southern sites were relatively mesic with more summer rainfall, whereas northern sites are climatically more extreme with less rainfall and greater seasonal temperature variation. Due to natural variation in reproductive activity, the number of flowering trees censused for lepidopteran damage varied by site and trip.

2.3. Lepidopteran caterpillar identification

Caterpillars were collected from infested syconia across the nine studied sites. We selected 14 caterpillars (at least one per site) and sequenced the mitochondrial gene cytochrome c oxidase subunit I (COI). We used primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA

TTG G) and HCO20198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Vrijenhoek, 1994) resulting in a total targeted mDNA region of 696 nucleotides. The targeted region was amplified by polymerase chain reaction with 35 cycles following this profile: 95°C for 30 seconds, 48°C for 30 seconds and 72°C for 90 seconds. We used NCBI BLAST (NCBI, R. C., 2016) to obtain a first approximation of closely related crambid taxa, finding that the lepidopteran antagonist of *F. petiolaris* clusters with members of the genus *Omiodes* (Guenée, 1854). To determine the position of our species within this genus, we constructed a COI phylogeny of *Omiodes* from our sequences and publicly available data from NCBI, including 3 sequences from *O. stigmosalis* associated with fig fruit in Costa Rica (D. Jansen, per. comm.), 1 sequences from *O. stigmosalis* collected by an amateur collector in Florida, United State (J. Hayden, per comm.) and 36 sequences from non-fig associated *Omiodes* (Haines and Rubinoff, 2012). Crambid species *Eurrhypara hortulata* and *Tetridia caletoralis* were included as outgroups, resulting a total of 46 sequences. After alignment using MAFFT (Katoh and Standley, 2013) and trimming using MEGA7.0.21 (Kumar et al., 2016), we obtained a final alignment of 604 nucleotides per individual. We built a phylogeny from this alignment using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The best model of sequence evolution was determined using FindModel (http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html). After setting the model to generalized time-reversible with gamma-distributed rate variation across sites and a proportion of invariable sites (GTR+Γ+I),, two simultaneous runs with 4 chains were executed for 10,000,000 generations, with a sampling realized each 1000 generations. Posterior probabilities were calculated with a discard of the first 1000 samples as burn in.

2.4. Lepidopteran damage data

Syconia attacked by lepidopteran larvae are easily recognized from the damage they cause. First instar larvae (3-6 mm in length) are aggregated in small groups (presumably siblings) on syconia where they generate a cluster small diameter holes chewed vertically into the fruit wall. Later instar larvae disperse to other syconia where they inflict increasingly greater damage, with fifth instar larvae (1.5-2 cm in length) consuming the contents of entire syconium. To obtain a tree-level measure of lepidopteran damage we sampled 15 independent syconia from each of four separate branches for a total of 60 fig fruits per tree. For smaller or less reproductive trees, we sampled as many syconia as were available. Each syconium was recorded as damaged or not damaged resulting in binary data set for each tree.

2.5. Measurement of fig reproductive phenology

To measure the phenological synchrony/asynchrony of syconia production within trees, we sampled 10 fruits from each of four branches per tree per trip. The syconia were opened and the developmental phase recorded. Seven development phases were recognized, two more than the A-E phases previously described by Galil and Eisikowitch (1968): pre-female phase (A phase: pistillate and staminate flowers immature), receptive female phase (early B phase: pistillate flowers mature, syconia attracting pollinating fig wasps), post-pollination female phase (late B phase: pistils decaying, syconia no longer attracting pollinators), early inter-floral phase (early C phase: early development of wasps and seeds), late inter-floral phase (late C phase: late development of wasps and seeds, pigmentation of larvae), male phase (D phase: staminate flowers and seeds mature, adult fig wasps present within the syconium), and post male phase (E phase: syconium expanding, softening, and sweetening, wasps have exited via hole chewed by

male fig wasps). We measured reproductive asynchrony within the crowns of individual trees using the inverse of Simpson's diversity index, which is a function of the observed proportion of syconia of each phase divided by the number of fig phases (Heip et al., 1998):

$$I = \frac{1}{\lambda} = \frac{1}{\sum_{i=1}^{R} p_i{}^2},$$

where I is evenness and our measure of reproductive asynchrony, λ is Simpson's index and p_i is the proportional abundance of flowering phase i. With seven phases recorded, the closer I is to one the more synchronous is the tree's reproduction (all the syconia sampled had the same developmental phase), and the closer the index is to seven the more asynchronous is the flowering (all flowering phases in equal abundance). Nonetheless, any $I \geq 1$ is indicative of within-crown asynchrony, while the maximum $I = 7$ requires perfectly even asynchrony that is unlikely to be observed in nature.

The size of a tree's crop was estimated from its level of reproductive activity and the volume of its crown. We followed Gates & Nason (2012) and estimated a tree's reproductive activity relative to its maximum fig production: 0, ≤ 5%, 5 – 25%, 25 – 50%, 50 – 75%, 75 – 95%, or ≥ 95%. The following formula was used to calculate the volume of an ellipsoid to estimate the size of a tree's crown:

$$V = \left(\frac{\pi}{6}\right) * a * b * c$$

with V being the volume of the crown, a the height of the tree, b the width, and c the length. We multiplied a tree's crown volume by reproductive activity to obtain a measure of its crop size.

2.6. Spatial aggregation of lepidopteran attack

If female Lepidoptera disperse locally to lay their eggs, then lepidopteran larvae and the associated damage of fig are expected to be spatially aggregated. We performed spatial autocorrelation analysis to investigate the spatial aggregation of lepidopteran fruit damage levels across trees within sites. Distance intervals (bins or lags) were determined for each site based on the local density of censused trees and ranged from 50 to 250 m. For each of our 24 site by study season data sets (Table 1), we then measured the mean correlation in the proportion of damaged fruit for each distance interval and plotted the mean for successive distance intervals as a spatial autocorrelogram. The significance of the mean correlation at each distance interval was determined by 1000 permutation replicates. These analyses were conducted in R (R Core Team, 2013) using the correlog function in the R package ncf (Bjornstad, 2009). Spatial aggregation is expected to result in significant positive correlations at smaller distance intervals followed fluctuating positive and negative values at larger spatial scales (Legendre and Fortin 1989). Consequently, in interpreting spatial autocorrelograms we focused on significant positive correlations at the smallest distance interval as evidence of spatial aggregation. Given our 24 site by season data sets, we conducted a total of 24 tests of damage correlation at the smallest distance. Assuming a Type I error rate of 0.05, under the null hypothesis of no spatial aggregation of lepidopteran fruit damage levels, we expected 0.025 x 24 = 0.6 of these tests to result in false significant positive correlations (and 0.6 tests to result in false significant negative correlations; 1.2 false significant tests total). Consequently, an observed number of significant positive correlations exceeding 0.6 was considered a global test of spatial aggregation of lepidopteran fruit damage across fig trees.

2.7. Modeling the ecological correlates of lepidopteran damage

We used Generalized Linear Mixed Models with binomial errors and a logit link function (logistic regression) to investigate the relationship between the odds of lepidopteran attack (binary counts: damaged/non-damaged fruits) and predictor variables *trees*, *seasons*, the interaction of *trees* and *seasons*, *sites*, *reproductive asynchrony*, *crop size*, and *tree density*. This analysis was conducted using the *glmer* function in the lme4 package (Bates et al., 2014) in R. The predictor variables *trees* and *trees by seasons* were treated as random effects because of the over dispersion of lepidopteran damage levels observed between trees within sites and because some of the same trees were sampled across field seasons. Two measures of *F. petiolaris* density were obtained, (i) the number of geo-referenced trees within a radius of 0.25 km, 0.5 km, 1 km and 2 km, and (ii) the distance to the 5th, 10th, 20th, and 30th nearest neighbor. The relative quality of alternative logistic regression models was evaluated by comparison of Akaike information criterion (AIC) scores, with an AIC difference of 10 or more considered a substantial difference in model support.

3. Results

3.1. Phylogenetic placement of lepidopteran fruit predator

Using NCBI Blast, COI sequences from the lepitopteran seed predator of *F. petiolaris* always matched best with *Omiodes stigmosalis*, most samples of which were obtained from fig fruit in Costa Rica. The sequences were 93% to 98% identical with *O. stigmosalis*, with lower scores likely due to ambiguous base calls in some sequences. Although the consensus phylogenetic tree contained some nodes with low support, three clades received high support: a Hawaiian clade, as seen in Haines and Rubinoff (2012), a clade comprised of two samples of

Omiodes diemenalis, and a clade including and all neotropical plus a few Australasian *Omiodes* (Figure 2). Within this latter clade, all fig-associated samples (from *F. petiolaris* and Costa Rica) form a single, well-supported monophyletic lineage. Further, based on posterior probabilities the *F. petiolaris* samples form a distinct lineage nested among previously sequenced *O. stigmosalis* samples, suggesting that *O. stigmosalis* is a single widespread species or closely-related set of species, in either case incorporating phylogeographic or host fig-associated genetic structure. We conclude from this phylogenetic analysis (and the natural history described above) that the lepidopteran predator of *F. petiolaris* is a fig specialist, and assume for the remainder of the paper that it is a genetically distinct population of *O. stigmosalis*.

3.2. Lepidopteran damage of figs

During the spring 2013 field season, 76 of 123 (62%) reproductively-active trees were attacked by *Omiodes* larvae (Table 1), with 14% of syconia being consumed (Supplemental Table 1). The following two field seasons showed a reduction in the proportion of damaged trees and syconia, with 86 of 189 (46%) of trees and 6% syconia attacked in fall 2013 and 73 of 236 (31%) of trees and 5% of syconia attacked in spring 2014. Lepidopteran larvae and damaged syconia were observed at every site during each season, however, most trees had little or no damaged syconia while only a few trees had high levels of damage, though in some cases with 100% of the fruit crop destroyed (Figure3). Site- and season-level data on the proportion of *Omiodes* damaged syconia are presented in Supplemental Table 1. The proportion of damaged syconia varied substantially across sites and seasons, ranging from nearly 0% to 40% (Figure 4). The highest damage was observed during the first field season (spring 2013) at Site 113 with an average 40% damaged syconia per tree and with 100% of syconia damaged on some trees. In

spring 2014, in contrast, only 5% of syconia were damaged at this site, illustrating the high variation observed between seasons at most sites (Figure 4).

3.3. Fig reproductive phenology

Measurements of reproductive phenology are presented in Supplemental Table 2. Across sites and seasons, the average percentage of reproductively active, fig-bearing trees was 42%, with more fruiting trees observed during the dry season (39% and 49% during spring 2013 and 2014) than during the wet season (38% during fall 2013). Our measure of within-crown reproductive asynchrony, I, averaged 2.06-2.35 over seasons, with a minimum of 1.29 at Site 179 in Fall 2013 and a maximum of 3.23 at Site 96 in Spring 2013. Though less than the maximum possible $I = 7$, asynchrony was ubiquitous and, indeed, 90% of the reproductive trees had syconia in two or more developmental phases. Reproductive trees varied greatly in estimated crown volume (347 m^3 with standard error 16.41) and, consequently, variation in crop size was large.

3.4. Spatial aggregation of lepidopteran attack

Spatial autocorrelation analysis of *Omiodes* fruit damage across trees was conducted for each site and season combination. Results of the 24 tests of autocorrelation at the smallest distance interval are presented in Supplemental Table 3. Of these tests, five were significantly positive, which is substantially more than the 0.6 tests expected to be false positives under the null hypothesis of no spatial aggregation lepidopteran damage. Only a single test was significantly negative (Site 158, November 2013), which is biologically insignificant given the expected 0.6 false-negative results. The five significant positive correlations were observed for different sites and seasons, with significant heterogeneity of spatial autocorrelation results

observed across seasons within sites. Results for Site 113 in central Baja are presented as an example of the spatial aggregation of *Omiodes* damage and its variation across seasons (Figure 5).

3.5. Ecological correlates of lepidopteran damage

Based on comparison of AIC scores, the preferred logistic regression model of *Omiodes* fruit damage included as fixed effects *seasons*, *sites*, as random effects *trees* and *trees by seasons*, and as covariates reproductive *asynchrony*, *crop size* and *tree density*. We tested the several measurements of *tree density* and obtained better fits with the distance to the 20^{th} nearest neighbor, which was used in all subsequent analyses. The AIC score for this model was 1559 and the results indicated a significant effect of the *crop size* (slope 0.77, $p = 0.0071$) and *tree density* (slope 0.14, $p = 0.0296$). The effect of *asynchrony* was marginally significant (slope -0.35, $p = 0.0503$). In an exploratory model, we replaced *reproductive asynchrony* with the *proportion of interphase syconia* (early + late) which had a significant positive association with fruit damage and resulted in substantially better support with an AIC score of 1548. This result makes biological sense as the syconia on which the larvae feed are primarily in interphase, and trees that do not have these syconia will experience little damage. Finally, we fit a model of *Omiodes* damage decomposing *crop size* into *crown volume* and *reproductive activity* as two separate predictor variables. This last, preferred, model had better support (AIC = 1542) and identified the *proportion of interphase syconia* (slope 1.72, $p = 0.0003$) and *crown volume* (slope 0.60, $p = 0.0005$) as having highly significant positive effects, and tree *density* (slope 0.12, $p = 0.061$) and *reproductive activity* (slope 0.86, $p = 0.091$as having only marginally significant positive effects.

4. Discussion

The main goal of this study was to identify the ecological factors influencing pre-dispersal damage of *F. petiolaris* syconia by the larvae of *O. stigmosalis*, an abundant and widespread lepidopteran species. To address this goal we measured fig tree reproductive characteristics, density, and fig damage rates at nine geographic locations spanning the range of the host species in Baja California. Before discussing the inferred relationship between predictor and response variables, we consider evidence supporting the phylogenetic placement of *F. petiolaris'* lepidopteran frugivore within the genus *Omiodes*.

4.1. Phylogenetic placement of lepidopteran fruit predator

Bayesian phylogenetic analysis incorporating publicly available *Omiodes* mitochondrial COI sequences and closely related crambid outgroups (Haines and Rubinoff, 2012), indicates that the lepidopteran predator of *F. petiolaris* fruits in Baja California forms a well-supported monophyletic clade. In turn, this clade is nested within a well-supported clade of *O. stigmosalis* reared from the fruit of Costa Rican *Ficus* (D. Janzen, pers. comm.) and from Florida (J. Hayden, pers. comm.). Given our taxon sampling, this suggests that fig-feeding *Omiodes* are a distinct Neotropical lineage. The moths reared from *F. petiolaris* differ in wing scale characters from described *O. stigmosalis* (Janzen, 1989; D. Janzen and A. Solis pers. comm.), suggesting they and *O. stigmosalis* may represent sister species whose biology is very similar. Further morphological and genetic studies would be necessary to confirm this hypothesis. Interestingly, we found that *O. stigmosalis* and the *F. petiolaris*-associated samples nested within the genus *Omiodes* with high support. This contradicts the suggestion of D. Janzen and A. Solis (pers. comm.) that *O. stigmosalis* is misplaced within *Omiodes* and that future taxonomic revision

would place it in a related crambid genus. Further phylogenetic analyses employing multiple loci and detailed morphological comparisons should help to resolve this issue. We also expect such analyses to resolve whether the *F. petiolaris* fig predator and *O. stigmosalis* represent a single species or closely-related species. For now, our working hypothesis is that *Omiodes* attacking the fruit of New World figs are all *O. stigmosalis*.

4.2. Lepidopteran damage of figs

Across sites and seasons, damage of syconia by *Omiodes* larvae varied greatly, occurring in 8 to 85% of *F. petiolaris* trees (Table 1) and in 0 to 40% of fruits (Supplemental Table 1). Overall, damage tended to decrease across the seasons, but with some exceptions (Figure 4). Site 158, for example, exhibited an increase in the level of damage over seasons, suggesting different lepidopteran population dynamics at the northern limits of the *F. petiolaris* range. *Omiodes* damage was observed on trees at all sites and censuses, mostly at low levels, though a few trees suffered complete crop failure due to pre-dispersal predation (100% damaged syconia). Thus, substantial spatiotemporal variation of fig damage was observed at the tree, site and season levels. Similar results have previously been reported for plant pre-dispersal seed predator systems (Ehrlén, 1996; Sperens, 1997; Leimu et al., 2002). As we report below, although the variation in fig damage does include a substantial stochastic component, it is associated with several predictor variables that, in turn, identify key ecological processes influencing interactions between *F. petiolaris* and *Omiodes* adults and larvae.

Bronstein (1988) reported unidentified moth larvae (likely *O. stigmosalis*) attacking up to 20% of syconia per crop of *F. pertusa* in Costa Rica. We observed a similarly low mean *O. stigmosalis* attack rate of 5-14% across seasons (Supplemental Table 1) in *F. petiolaris*, however

we also occasionally observed outbreak dynamics in which 100% of syconia within a crop were infested. The difference in the incidence of these outbreaks between Bronstein's and our study may be a consequence of sample size, with 555 fruit crops sampled from *F. petiolaris* versus 14 from *F. pertusa*. As we have observed for *O. stigmosalis* associated with *F. petiolaris*, Bronstein also reported larvae moving from fruit to the ground, where they likely pupated in the soil. More recently, Sugiura and Yamazagi (2004) reported larvae of two moth species (*Pachybotys spissalis* [Crambidae] and *Stathmopoda sp.* [Oecophoridae]) infesting 0-38.5% of syconia sampled from six *Ficus* species (1-3 trees per species) on Iriomote Island, Japan. Moth larvae attacking figs are thus not limited to North American *Ficus* and may have broad-scale implications for the stability and fitness of fig-pollinator mutualisms. In the following section, we discuss ecological factors statistically associated with patterns of *Omiodes* damage in *F. petiolaris*.

4.3. Lepidopteran damage response to tree density and reproductive characteristics

Logistic regression analyses indicate that attack of *F. petiolaris* fruits by *Omiodes* larvae is positively associated with the production of larger, more synchronous fruit crops and, to a lesser extent, the local density of fig trees. The positive effect of *crop size* is consistent with our hypothesis that the larger the number of syconia on a tree, the more it attracts ovipositing moths and the more fruit damage it experiences. An additional explanation for the positive effect of *crop size* is that larger crops increase the likelihood that larvae will successfully locate successive syconia, as required for their development. As a consequence, larger crops may experience greater fruit damage not because they host more *Omiodes* larvae, but because individual larvae are able to destroy more syconia. While we found a significant relationship

between *crop size* - the estimated number of syconia on trees - and *Omiodes* damage, this estimate was obtained as the product of *crown volume* and *reproductive activity*, and when analyzed separately, *crown volume* was significant whereas *reproductive activity* was not. These observations suggest that tree size, and not just fruit production, may be important in attracting ovipositing *Omiodes* and supporting larval development.

Predator satiation is an anti-predator adaptation in which prey (or fruit) occur at high density only sporadically, reducing the probability of individual prey being eaten (Janzen, 1971; Silvertown, 1980; Kelly & Sork, 2002; Shibata et al., 2002; Espelta et al., 2008). Satiation of seed predators is often associated with population-scale masting, however sporadic reproduction can also occur at the individual tree level and locally satiate predators (Nilsson and Wastljung, 1987). Although we find fruit damage to be positively associated with *F. petiolaris* crop size, predator satiation is suggested by only a minority of available syconia being attacked by *Omiodes* larvae. Moreover, our observation that most reproductive trees have low levels of syconium damage suggests that the sizes of *Omiodes* populations associated with *F. petiolaris* are not limited by the availability of fig syconia.

In contrast to the large majority of fig species in the moist and wet tropics that bear highly synchronized fruit crops within trees, 90% of the reproductively active *F. petiolaris* trees we censused had syconia in two or more developmental phases. That attack of *F.* petiolaris fruits by *Omiodes* larvae is negatively associated with within-tree reproductive asynchrony does not support our hypothesis that asynchronous crops and greater phenological overlap of syconia and insect antagonists increase the likelihood of moth oviposition and, consequently, larval damage of fruit. A potential explanation for this unexpected observation is that greater asynchrony decreases the relative abundance of interphase syconia, which is the developmental stage most

susceptible to *Omiodes* attack (F. Piatscheck, pers. obs). Also relevant is the behavior of *Omiodes* larvae to damage several syconia during their development, consuming the content of an interphase syconia and then moving to a new healthy one by night. When fruit are produced asynchronously within a tree, these susceptible syconia are fewer and located farther apart, which may decrease the odds of their being located and predated. Consistent with this explanation, our favored logistic regression model revealed a significant positive relationship between the level of *Omiodes* damage within a crown and the proportion of developing syconia specifically in interphase.

Plant density has been associated with increased herbivory (Bach, 1988), decreased herbivory (Platt et al., 1974) or found to have no effect on herbivore dynamic (Bach, 1980). In *F. petiolaris*, *tree density* was marginally significantly ($p = 0.061$) associated with greater *Omiodes* damage, which may help explain the spatial aggregation of fig damage observed in some sites and seasons (Figure 5). These two results emphasize the need to examine ecological phenomena at a range of spatial scales. Because of the association of *F. petiolaris* with rocky substrates, its populations are patchily distributed and often have high local tree densities, both of which contrast with figs typical of moist and wet tropical environments. Given this unusual population structure, it is possible that the positive association between *Omiodes* damage and *F. petiolaris* density will not generalize to the majority of Neotropical fig species.

Other, unmeasured ecological factors may also affect lepidopteran activity. For example, Jandér (2015) examined the effects of ants on *F. obtusifolia* and its associated pollinator, finding that ants reduce fig predation by an unidentified lepidopteran caterpillar. Ants have also been observed on *F. petiolaris* and could play an as yet unappreciated role in limiting in *Omiodes*

damage. That said, on *F. petiolaris*, ants are largely absent at night when *Omiodes* oviposit and larvae disperse to locate new fruit.

4.4. Fig reproductive phenology

We observed substantial variation in *F. petiolaris* reproductive phenology across the nine study sites and three extended field trips spanning two seasons. Despite this variation, fig production was observed at each site during each visitation, consistent with the pattern of year-round fruiting that is characteristic of fig species. More interesting is that most trees exhibited within-crown asynchrony in reproductive phenology, contrasting with the highly reproductive synchrony characteristic of fig trees in moist and wet Neotropical forests (Windsor et al., 1989). Within-tree asynchrony was initially thought to be an adaptation to extreme seasonality enabling pollinator generations to cycle within the natal tree and figs to reproduce via selfing (Bronstein, 1989). Bronstein (1992) later suggested that this asynchrony may also be an adaptation promoting the overlap of reproductive activity and outcrossing between trees, which was demonstrated to be the case in *F. petiolaris* (Gates & Nason, 2012). Smith & Bronstein (1996) reported within-tree reproductive asynchrony in *F. petiolaris* at two locations in mainland Sonora, Mexico. In our study of nine locations in Baja California, we found reproductive asynchrony to independent of tree size, which was also independent of reproductive effort; we observed small trees with heavy crops, large tree with light crops, and vice versa. Our results also generalize the distribution of asynchrony in *F. petiolaris*, revealing it to be very common across the range of the species in Baja California, including more mesic areas to the south and more xeric areas to the north. *Ficus petiolaris* adds to the list of fig species exhibiting within-tree asynchrony (Bronstein & Patel, 1992; Cook & Power, 1996; Yu et al., 2006; Lin et al., 2008;

Yang et al., 2014). Yang et al. (2014) demonstrated that *F. microcarpa* trees from the same genetic group (clone) had similar flowering characteristics, including within-tree asynchrony, suggesting for the first time a genetic basis for within-tree asynchrony in *Ficus*. Further studies would be useful to understand the degree to which genetic relatedness, spatial proximity, and similarity of microsite conditions influence variation in patterns of *F. petiolaris* flowering and successful pollinator and pollen dispersal between trees.

5. Conclusion

In this study, we provide a survey of lepidopteran pre-dispersal seed predation across sites and seasons and identify ecological factors that influence lepidopteran damage at the tree level in *F. petiolaris*. We found the syconia borne by larger trees and in denser patches to be more susceptible to attack by *Omiodes* larvae, which in turn can have severe consequences for the production of seeds and mutualist fig wasp pollinators, occasionally including the loss of entire fruit crops. In contrast, we found within-tree reproductive asynchrony to be negatively associated with *Omiodes* damage. Within-tree asynchrony and small fruit crops are common in *F. petiolaris*, though unusual of fig species in general. It has previously been suggested that asynchronous fruiting at the tree level and year-round fruiting at the population level may be adaptations to extreme environments, such as deserts, that favor the maintenance of obligate pollinator populations and fig reproductive success (Janzen, 1979; Bronstein, 1989; Gates & Nason, 2012). As revealed by this study, however, tree-level asynchronous fruiting also appears to benefit the *F. petiolaris*-pollinator mutualism by decreasing predation of seeds and larval pollinators by *Omiodes* larvae. A better understanding of the adaptive significance of

reproductive synchrony/asynchrony in figs will be provided by future studies disentangling the relationship between variation in this trait and different sources of fig and fig wasp fitness.

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Table 1. Site information from north to south, including numbers of mapped trees and incidence of *Omiodes* damage on flowering trees by study season (S denotes spring [May-June], F denotes fall [November-December]).

Site	Latitude, longitude	No. mapped trees	No. damaged trees/no. flowering trees		
			S2013	F2013	S2014
Site 158: Cañon Aguajito Higuera	29,2628391, -114,021134	103	8/19 (42%)	10/18 (56%)	17/20 (85%)
Site 172: Bahia San Francisquito	28,26775278, -113,1095672	70	9/14 (64%)	6/14 (43%)	7/15 (47%)
Site 112: Sierra San Francisco	27,56043406, -113,0671864	75	9/15 (60%)	7/21 (58%)	10/41 (23%)
Site 113: La Higuera	27,0995915, -112,4968451	76	15/20 (75%)	13/24 (54%)	11/30 (37%)
Site 95: Fig Canyon	26,3598798, -111,8040866	101	N/A	14/26 (54%)	9/29 (31%)
Site 179: Nopolo	25,91341244, -111,3514215	38	N/A	4/13 (31%)	2/19 (11%)
Site 201: El Ranchito	25,37795264, -111,3125629	42	N/A	4/7 (57%)	2/16 (13%)
Site 96: La Paz Summit	24,0459034, -110,1344447	337	11/18 (61%)	14/29 (48%)	3/39 (8%)
Site 70: San Bartolo	23,73747426, -109,8275927	105	24/37 (65%)	14/37 (38%)	14/34 (41%)
TOTAL		947	76/123 (62%)	86/189 (46%)	75/243 (31%)

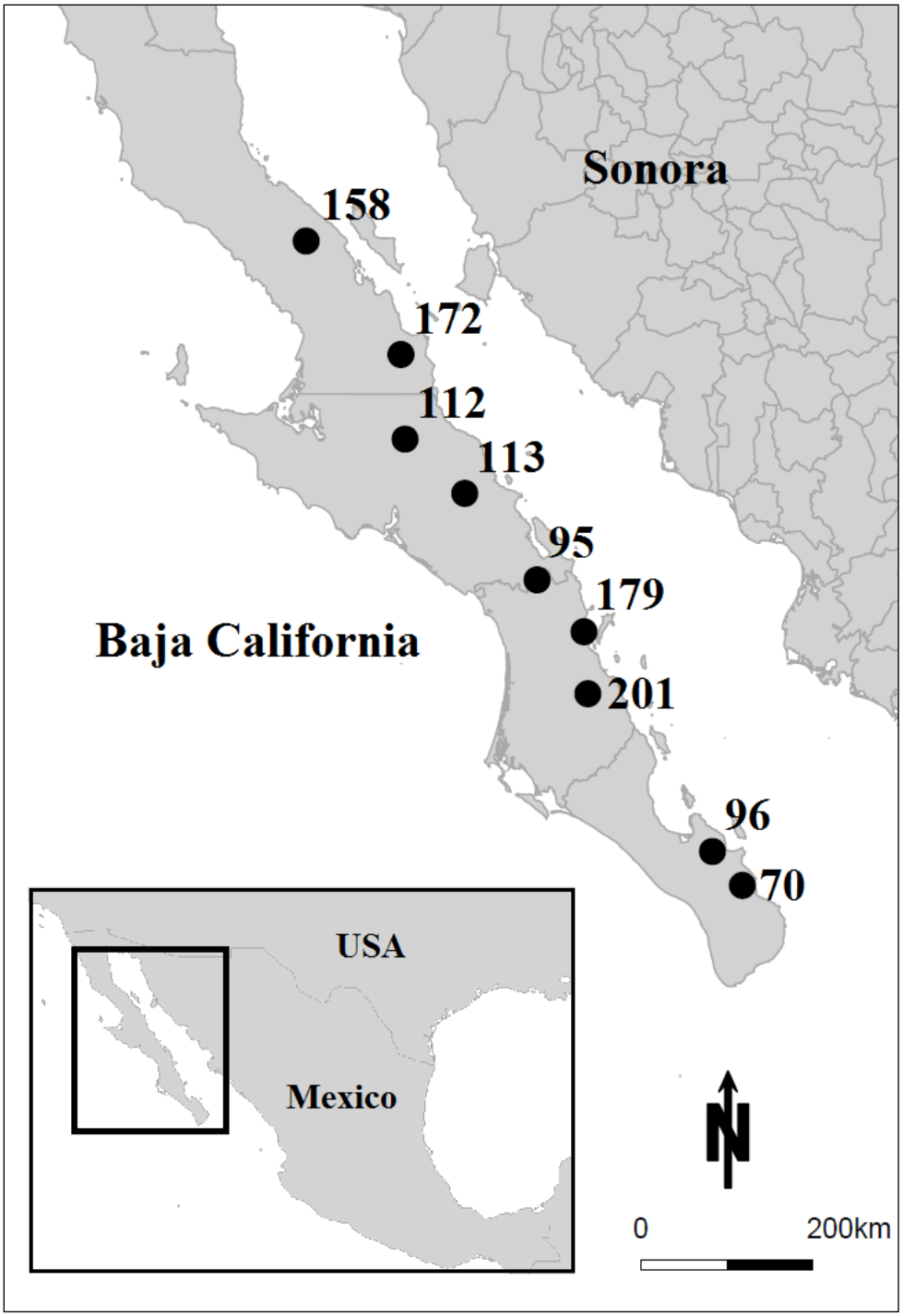


Figure 1. Locations of the nine *Ficus petiolaris* study sites located along the Baja California peninsula, Mexico. The numbers correspond to the site designations described in Table 1.

Figure 2. Consensus tree from Bayesian phylogenetic inference of the pre-dispersal predator of *F. petiolaris* seeds and wasps, *O. stigmosalis* reared from Costa Rican figs, and 38 *Omiodes* sequences from Haines and Rubinoff (2012). Values above the nodes represent posterior probabilities. Values below 0.5 are not shown and associated nodes are collapsed.

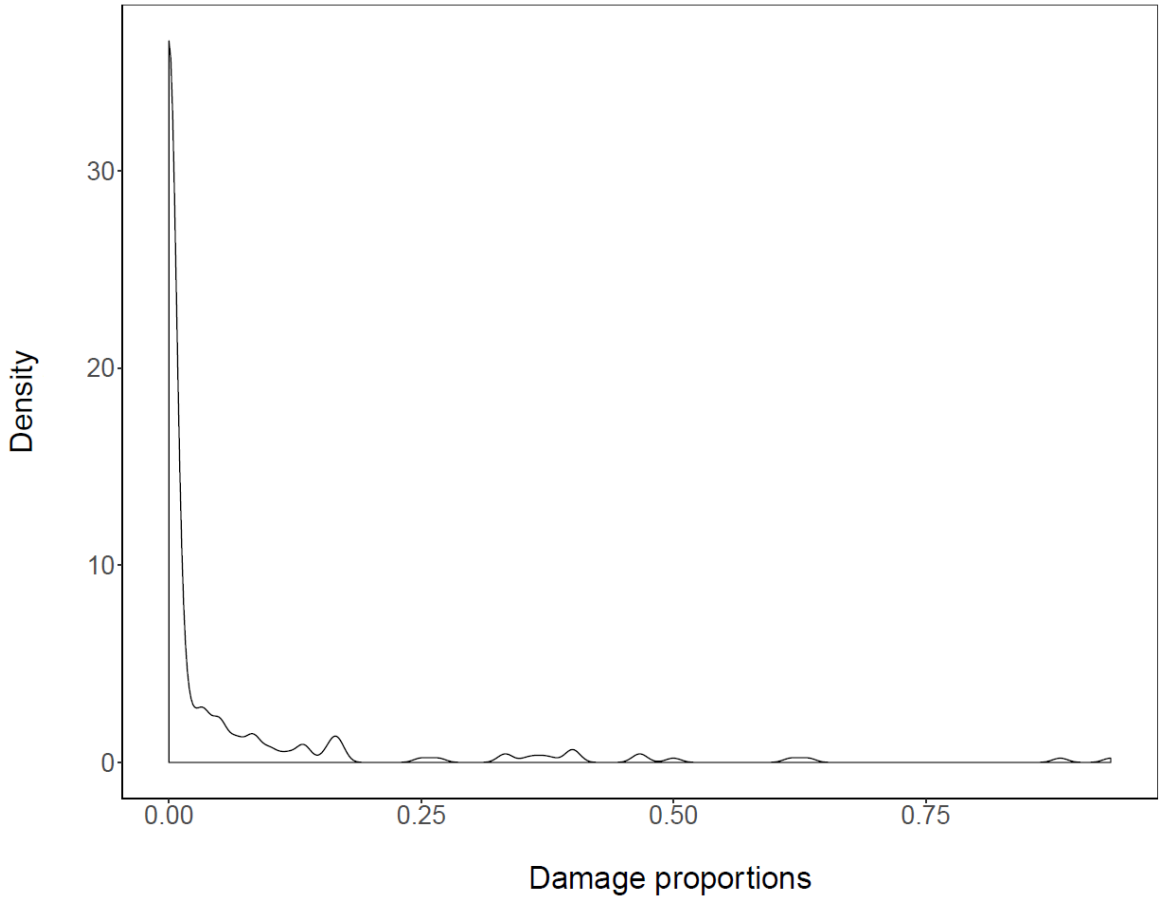


Figure 3. Density plot of the proportion of *Omiodes*-damaged syconia, with data pooled across trees, sites, and seasons.

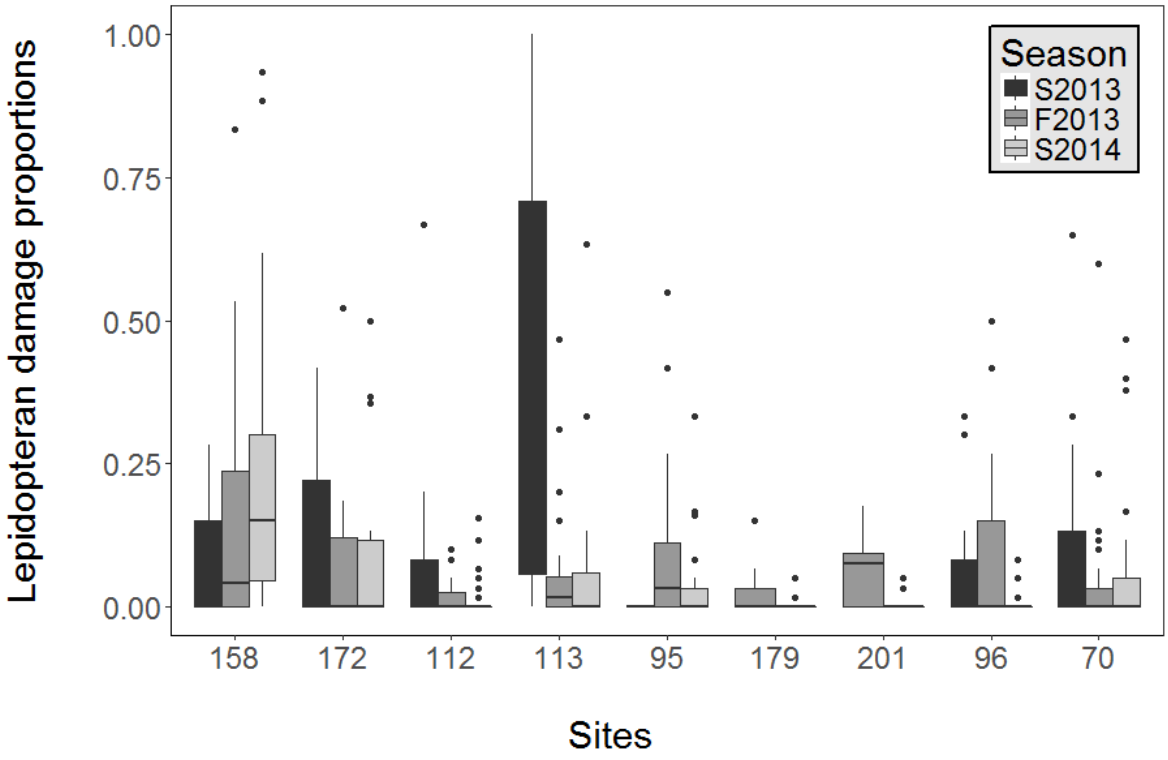
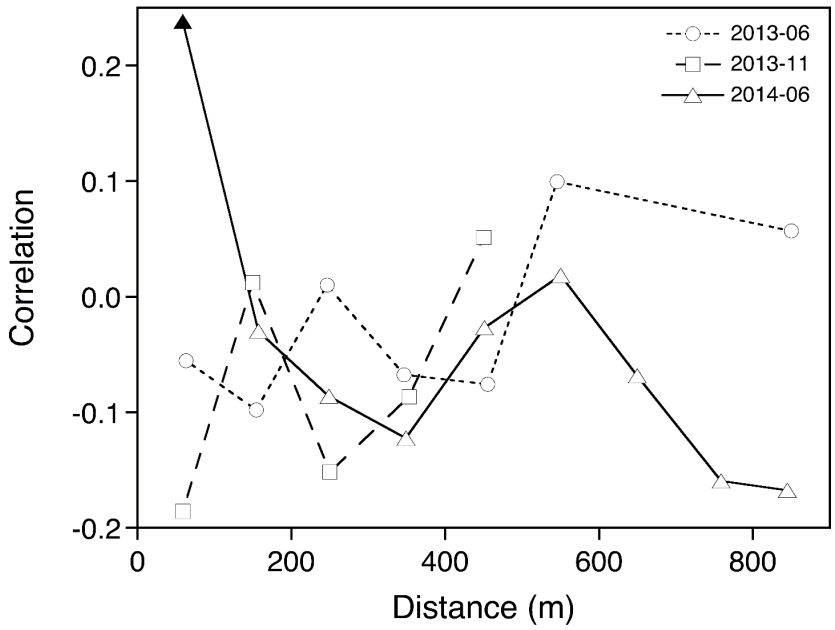


Figure 4. The proportion of *Omiodes* -damaged fig syconia across sites and seasons. Sites are designated as in Table 1 and are ordered from north (158) to south (70).

A



B

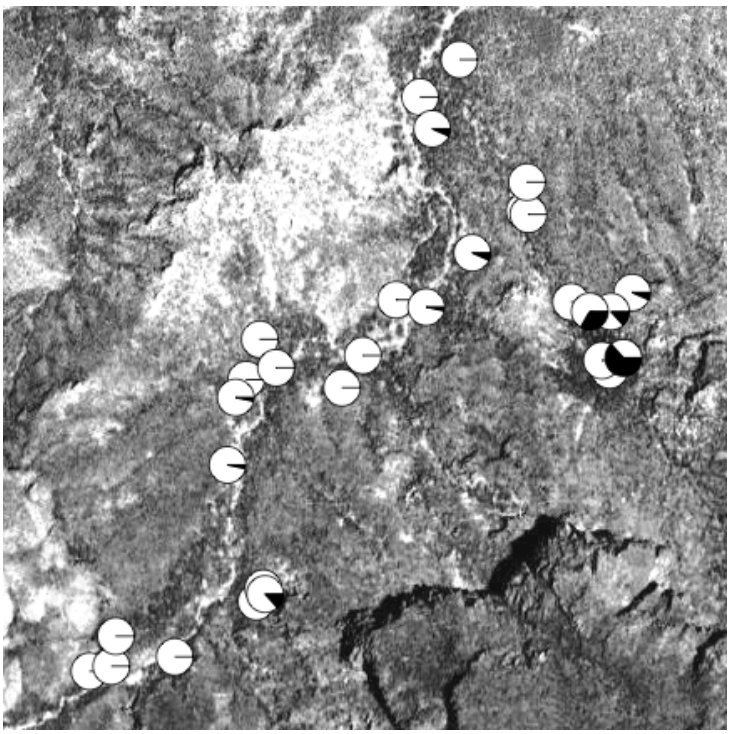


Figure 5. Spatial aggregation of *Omiodes* damaged *F. petiolaris* syconia at Site 113. A) Spatial autocorrelograms of damage at 100 m distance intervals for three seasons (significant correlation indicated by filled symbols). B) The proportion of damaged (black) and undamaged (white) syconia mapped to trees at Site 113 for May 2014, the season with significant autocorrelation of damage at the smallest distance interval, as shown in panel A. Map image is 1 km square.

CHAPTER 5. CLIMATE CHANGE EFFECTS ON MUTUALISM: A PROJECTION OF SPECIES INTERACTIONS INTO THE FUTURE

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Abstract

Species communities are dynamic through responding differentially to changes in their environment. Forecasting changes in species community structure and dynamics is a challenging but necessary task to understand human-driven climate change impact on species interactions, and for biodiversity purposes in general. Highly interdependent species and, mutualisms in particular, are at risk because the negative impact due to environmental change on one species will directly impact the species that depend on it. Species distribution modeling and projection under future climate scenarios has been increasingly used to predict species' habitat suitability or distribution in response to changing climate but often lack taking species interaction into consideration. In this study we modeled the association of the abiotic environment with fig wasp community composition (one mutualist and height parasites) associated with *Ficus petiolaris*, an endemic fig tree of Mexico. Combining ecological modeling and species distribution modeling, we show that near-future climate change will likely expand *F. petiolaris*' habitat suitability

northward but could be associated with a reduction of pollinator production to the benefit of parasites, thus reducing the fitness of both host plants and pollinators. This approach allowed us to model species interactions into the future and is an example that shows that species distribution modeling alone is not enough to measure the impact of future climate change on species.

1. Introduction

Ecological forecasting is becoming an imperative to understand the immediate impact of global change on ecosystems and for policy decision making (Clark et al., 2001; Dietze, 2017). This new discipline aims to study ecological processes and make predictions on potential change their functioning in the near future, in order to make scientific studies relevant to the necessity of understanding the effects of current global change and environmental degradation (Dietze et al., 2018). Although ecological forecasting studies have often focused on large scale natural and agricultural ecosystem response to climate change (Cramer et al., 2001; Tilman et al., 2001; Knapp et al., 2002; Helmuth et al., 2006; Craft et al., 2009; Schlenker & Lobell, 2010), attempts to forecast ecological processes at the community level in the near future are relevant and consistent with our urgent need of understanding human-mediated global change impacts on biodiversity (Botkin et al., 2007; Suttle et al., 2007; Diez et al., 2012). Here we present a study in which we attempt to forecast the community dynamic of a multipartite obligate mutualists-antagonist system in response to climate change.

Mutualism interactions are ubiquitous in nature and play essential roles in ecosystem functioning and evolution of communities (Bronstein, 2015). Changes in their biotic and abiotic environment can have differential consequences on their interaction resulting in a multitude of

possible outcomes (Dunn et al., 2009. Yang & Rudolf 2010; Kiers et al., 2011). However, today mutualisms are under threat: human mediated global change is thought to have dramatic consequences on species interactions, especially mutualism (Bronstein et al., 2004: Tylianakis et al., 2008; Dunn et al., 2009; Yang & Rudolf 2010). Although disruption of a mutualism doesn't necessarily mean species extinction, the loss of any mutualist should have direct consequence on its partners. The consequences of anthropogenic changes are thought to be even more intense on obligate mutualisms, which is the case when interacting species require their mutualistic partner to live or reproduce (Bronstein et al., 2004). The loss of their mutualists has in these cases immediate aftereffect leading fatally to coextinction (Dunn et al., 2009; Wernegreen, 2012). This problem is considerable, especially that mutualisms have been shown to promote diversity (references), fulfill essential ecosystem services (reference), stabilize ecosystems and to minimalize coextinction (references, Fricke et al., 2017). Plant-pollinators, in particular, are omnipresent in natural and agricultural systems and have been on the decline for the last decade (Burkle et al., 2013). This has already important effects on ecosystem functioning and services and is predicted to negatively impact human wealth and security (Potts et al., 2010).

Mutualisms do not occur in isolation and are often subject to exploitation by antagonists (Bronstein, 2001). Parasites are no exception, and many are threatened by climate change (Dunn et al., 2009). Global change is particularly expected to affect species that have high dependency with each other (i.e., symbiotic, specific, obligate and vertically transmitted interactions). The loss of a mutualist will have immediate repercussion on the species that have high dependency with it. Currently, the rate of species extinction is unpreceded (Barnosky et al., 2011; Ceballos et al., 2017) and it is vital to understand the global change impact on species interactions and their repercussion on biodiversity and ecosystems functioning.

Here we aim to model the consequences of climate change on species interaction dynamics. Species distribution models are often used for ecological forecasting and conservation purposes (Lawler et al., 2011) but often fail to take species interaction into consideration (Urban et al., 2016). In a previous study we studied the association between climatic variation (i.e., temperature and precipitation) and a fig wasp community reproductive dynamic, in obligate interaction with fig trees (Piatscheck et al., unpublished data). In this system, one specialized chalcid wasp species is mutualist with its fig host (*Ficus*¸ Moraceae) and provides it with the service of pollination and eight specialized parasitic wasp species (i.e., non-pollinating fig wasps) exploit the mutualism by utilizing resources normally attributed to fig tree or pollinating wasp reproduction. Pollinator wasps oviposit into flowers from within the syconia (thus are protected from extreme environment). Non-pollinating fig wasps remain most of their adult life outside the syconia, thus more exposed to temperature and precipitation extreme, potentially lethal to them. Thus, we hypothesized that high temperature and increased precipitation would negatively impact both pollinator wasps and non-pollinating fig wasps, but would have a more negative impact on non-pollinating fig wasps which in turn advantages pollinators because they have fewer parasites,

We tested this hypothesis by modeling the logarithm of the odds of the reproductive success of pollinating wasps relative to the success of non-pollinating fig wasps and found a positive association between an increased proportion of pollinators with both an increase in temperature and precipitation in Baja California (Piatscheck et al., unpublished data). As temperature is predicted to increase, rainfall to decrease and extreme events potentially being more frequent in Baja California (Cavazos & Arriaga-Raminez, 2012; Vaghefi et al., 2017), be predicted a potential benefit for the mutualism from increased temperature but also an eventual

disadvantage due to reduced rainfall. In this study, we use this knowledge to investigate the change in climate in Mexico with future climate scenario averaged around 2070. We later use species distribution modeling to predict the future habitat suitability of the host tree and project the fig wasp dynamics on the landscape into the future.

2. Materials and methods

2.1. Study system

Fig-fig wasp mutualisms are cases of nursery mutualisms, in which trees in the genus *Ficus* evolved a reproductive strategy that requires specific chalcid wasps (order: Hymenoptera; superfamily: Chalcidoidea) that enter the fig inflorescence (i.e., the fig fruit, or syconium) and provide pollination services and at the same time extract a cost to the fig: the infestation of some flowers with wasp eggs that will develop inside the syconium (Janzen, 1979). This mutualism is obligate, meaning that the partners need each other in order to reproduce. Fig-fig wasp mutualisms are associated with specialized non-pollinating fig wasps which are obligate antagonists to the system. Their feeding biology varies, from competitors with the pollinators for flower resources, to parasitoids of other fig wasp species (Borges, 2015) and have been shown to have negative impact on the pollinator production (i.e., the male function of the fig tree by pollen dispersal) and seeds production (Kerdelhué & Rasplus, 1996; West et al., 1996; Conchou et al., 2014; Castro et al., 2015). Fig trees have important ecological roles and are often considered keystone species, especially in the tropics (Terborgh, 1986; Lambert and Marshall, 1991; Shanahan et al., 2001; Harrison, 2005; Kissling et al., 2007).

Ficus petiolaris Kunth is a fig species endemic to Mexico and is widely distributed from Sonora to Oaxaca, and in Baja California (Serrato et al., 2004; Ibarra-Manríquez et al., 2012). It

is pollinated by one species of *Pegoscapus*, and is the host of eight chalcidoid non-pollinating fig wasp species: three species of *Idarnes*, two species of *Heterandrium*, one species of *Ficicola* and its potential parasitoid, a species of *Physothorax*, and, finally, in rare instance a species of *Sycophila*. The biology of each of these species is not clearly known yet, but the most common non-pollinating fig wasp is a species of *Idarnes* is probably a competitor with the pollinator while the other *Idarnes* which belong to the group *carme*, could potentially be cleptoparasite of *Pegoscapus* (Piatscheck et al., unpublished data). The proportion of these parasites within syconia can be tremendously high, up to 97% (Piatscheck et al., unpublished data).

2.2. Wasp community modeling with local climate

During a previous study, we collected 2367 syconia from nine populations of *F. petiolaris* during four collecting trips (Piatscheck et al., unpublished data). The wasps were sorted and counted by species for each syconia. Within-syconia fig wasp community structure was highly variable across sites and seasons (Piatscheck et al., unpublished data). We later measured a series of biotic and abiotic variables and tested their association with fig wasp counts. Air temperature data were estimated with MODIS' land surface temperature derived from satellite imagery (MOD11A1 data set, Wan et al., 2015). Land surface temperature and air temperature have been shown to be associated (Gallo et al., 2011) even though their association varies weather the sky is cloudy or clear (Gallo et al., 2011), or depending on the land covers (Mildrexler et al., 2011). Here we use it as a proxy to estimate variation in air temperature, knowing that land surface temperature is usually 2–8°C above air temperature (Gallo et al., 2011). The authors later used mixed effects logistic regressions to highlight potential

associations between environmental variables and the odds of pollinator success relative to the odd of the non-pollinating fig wasp success as follow:

$$\log\left(\frac{Pi}{1-Pi}\right) = \ ß_0 + \ ß_1X_1 + \ ß_2X_2 + \ ß_3X_3 + \cdots + \ ß_nX_n$$

with P_i the odd of success of the pollinator calculated from pollinator and non-pollinating fig wasp counts, $ß_0$ is the intercept and $ß_n$ are the coefficients associated with the environmental variables X_n. The models were conducted using the *glmer* function in the lme4 package (Bates et al., 2014) in R. To equalize the contributions of predictor variables in the linear mixed models, all were standardized to reduce their range from 0 to 1 (x = (x – min(x) / (max(x) – min(x))).

2.3. Occurrence data

Occurrence data consisted of an initial 3329 occurrence points after removal of ambiguous or erroneous data, obtained by the Nason lab and database coordinates from GBIF, iDigBio and iNaturalists extracted with *rgbif*, *spocc* and *rinat* in R (R Core Team, 2019) respectively (Chamberlain et al., 2016; 2018; Barve & Hart, 2014). Bias correction and removal of duplicates resulted in a data set of 140 occurrence points with coordinates occurring in Mexico only.

2.4. Current and future environmental variables

Current global environmental averages for 19 bioclimatic variables, as well as elevation, were obtained for Worldclim v2 at the 2.5° x 2.5° resolution (Fick & Hijmans, 2017). Soil properties were obtained from Soilgrid, at a 5km x 5km resolution (Hengl et al., 2017). Detail of all the abiotic variables obtained for this study are presented and described in Table 1. Because correlated variables might create a bias in the species projection outcomes, it is recommended to

select a subset of environmental variables, so we selected variables with Pearson correlation coefficient < 70%. Correlation between initial variables can been seen in Supplemental Figure 1, which was realized with the R package *corrplot* (Wei et al., 2017), and retained variables for downstream analyses are noted in Table 1. When arbitrary choices needed to be made, we selected the variable that seemed the most relevant to the biology of our model.

Future climate bioclimatic data were obtained from Worldclim v1.4 at the 2.5° x 2.5° resolution (Fick & Hijmans, 2017). They consist of data from the Coupled Model Intercomparison Project Phase 5 and propose bioclimatic data from different general circulation models (GCM). These data being mostly available for two Representative Concentration Pathway (RCP), we sectioned data from all the GCM models for RCP 4.5 and 8.5 predicted for the year 2070. All the bioclimatic variables of all the GCM models were then averaged for both RCP scenarios, in order to create one ENSEMBLE scenario that summarizes all available GCM models. For future climate data, elevation and soil variable were considered being the same.

2.5. Species distribution and wasp interaction forecasting

We used with the package *sdm* (Naimi & Araujo, 2016) to train several models: GLM (Nelder & Wedderburn, 1972), GAM (Hastie & Tibshirani, 1990, GBM (Friedman, 2001), We used with the package *sdm* (Naimi & Araujo, 2016) to train several models: GLM (Nelder & Wedderburn, 1972), GAM (Hastie & Tibshirani, 1990, GBM (Friedman, 2001), SVM (Cortes & Vapnik, 1995), Random Forest (Breiman, 2001), MARS (Friedman, 1991). and Maxent (Phillips et al., 2006). Fitted models were evaluated using 30% of the initial dataset to test the trained model for three iterations. The models' performances were then judged given several criteria: percentage of occurrences fitted in the model, mean AUC (Fielding & Bell, 1997) and mean TSS

values (Allouche et al., 2006). Both these statistics indicate better performance of the model at higher values (range: 0-1). Because it has been also criticized that species distribution models can provide very different results and make it difficult to choose which model to use, we follow the recommendations that we should the ensemble of the models for suitability projection (Shabani et al., 2016). Thus, we retained all models that were successful and created a weighted model by the TSS scores and used it to project the present habitat suitability. The threshold of presence of *F. petiolaris* on the map was selected with the maximum of sensitivity plus specificity, where the sensitivity is the percentage of actual presences predicted and the specificity is the percentage of actual absences predicted. The threshold values of all models were considered, and the average was calculated. This value was then utilized to create a presence/absence map of the projected distribution of *F. petiolaris.*

From the logistic regression analysis, intercept and coefficient values were used as a model to project logarithm of odds of pollinator relative to the odds of non-pollinating fig wasps as follow:

$$\log\left(\frac{odd\ pollinator}{1 - odd\ pollinator}\right) = -2.82 + 1.70 * Temperature + 3.23 * Precipitation$$

Because all the variables were transformed while modeling the mixed effect logistic regressions, we standardized future temperature and precipitation from 0 to 1 with x = (x – min(x) / (max(x) – min(x)). Then, we used the model to project fig wasp dynamic with temperature and precipitation over the predicted distribution of *F. petiolaris* to represent change in the community associated to their host.

3. Results

3.1. Variable selection model evaluation

From the 35 environmental variables selected for distribution modeling, a substantial set of these variables showed correlation higher than 0.7 (Supplemental Figure 1) and we retained 14 of them (Table 1). Nine of them were bioclimatic variables and seven were soil property data. The importance of these variables was different across all models (Figure 1), but Mean Temperature of Driest Quarter (Bio9) had a relatively high importance for all models. All models performed well with predicting habitat suitability on 100% of the occurrence data (Table 2). From the evaluation statistics, the model that performed the best was Random Forest, followed by MAXENT. GBM performed the worst. We later used a weighted ensemble for habitat suitability projection.

3.2. Future habitat suitability

The threshold value obtained with the maximum of sensitivity plus specificity was 0.14. Thus, values above 0.14 represent the habitat suitability projected of *F. petiolaris* (Figure 2). Projected future suitability show an expansion towards the north-east from its current position (Figure 2). The environmental conditions seem to be even favorable in some areas in the highlands of the Mexican Plateau. In Baja California, the suitability seems to follow the same trend with a small northward expansion. Both RCP 4.5 and 8.5 showed the same tendencies with RCP 8.5 shifting the suitability even more towards the north and increase the area of suitability. Globally, future climatic conditions seem to favor *F. petiolaris* because the suitability at *F. petiolaris*' current location seem to remain favorable in the near future (except, it seems, along the southern coast of Mexico) and even expands toward the north.

3.3. Wasp community dynamic projection

As shown in Figure 3, the temperature is expected to increase in both RCP scenarios as well as precipitation that is expected to decrease drastically. The temperature will increase especially in the northern part of Mexico, whereas precipitations are especially expected to be reduced in the southern part of Mexico, in current location of rainforests. The projection of the log-odds of pollinators relative to non-pollinators on the future "presence" of *F. petiolaris* (i.e., future suitability past the presence threshold) is always below 0, indicating higher odds of non-pollinating fig wasp than pollinators. The log-odds values are however varying, with higher prevalence of non-pollinating fig wasps in Baja California, north Sonora and east Oaxaca, indicating for the zones where more non-pollinating fig wasps are expected.

4. Discussion

This study attempted to project species interactions into the future, with a fig–fig-wasp community as an example. First, the projection of habitat suitability for *F. petiolaris*, and its potential distribution is moving northward, a trend that is already observed in other organisms as a response to climate change (Parmesan & Yohe 2003; Root et al., 2003). *F. petiolaris* is particularly expected in the north of Sonora, expanding eventually in the United States in the future. We expect however that trend to be limited as dispersal is a process which is not as fast as habitat climate change and was not considered in these models (Pearson & Dawson, 2003).

The projection of the fig wasp odds is somewhat unexpected. As temperature is positively associated with higher production of pollinators but negatively associated with reduced rainfall and given the increase of temperature predicted in the future (Figure 3), we expected a balance between zones of advantage for the pollinators and zone of advantage for the non-pollinating fig

wasps. However, the whole area where *F. petiolaris* is predicted in 2070 shows negative values, suggesting climatic conditions favoring the non-pollinating fig wasps, thus, detrimental for the mutualisms. It is interesting because while not taking the host' future suitability, projection of the interaction indicated zones favorable for the pollinator (i.e., >0, data not shown). However, none of these zones are within the future habitat suitability of the fig tree. The projection shows some variations though, indicating more favorable conditions for the pollinator in Sinaloa, Gerrero and Michoacán. Unfavorable zones are interestingly located at the limits of range of *F. petiolaris*. We can predict that new colonizer toward the north might be more associated with non-pollinating fig wasps, thus limiting their fitness.

We are aware of the limits of species distribution modeling into the future, as our models do not take into account dispersal, other biotic interactions (as competition), and potential evolutionary adaptation to changing climate variables (Dormann, 2007). However, this approach indicates interesting aspects that could improve future species distribution modeling. From the pollinating fig wasp perspective, modeling the host future range is crucial because the wasp rely on it to survive. Taking into account both the host distribution and its interaction with antagonist insects reveal its projected dynamic relative to the parasites. Second, from the fig tree perspective, this approach indicates where its fitness might be limited. In *F. petiolaris*' case, the zones where it is predicted to expand are predicted to be associated with more non-pollinating fig wasps, suggesting a potential limited colonization rate toward the north, due to high level of parasitism.

5. Conclusion

Combining both ecological modeling and species distribution modeling reveal here some interesting aspect for forecasting the future of species interactions. This approach implies rigorous observation of species dynamic considering spatial and temporal variation of the species interactions, with their associated climate variables. To our knowledge, no one has attempted to model species interactions changes into the near future, combining the host future species distribution and the dynamic of its interacting species. This method represents an opportunity to fulfill the lack of biotic interaction usually absent in species distribution models and predict climate change effects on species from a community perspective. Our approach of modeling the interaction of plants and their pollinator could be reproduced to other biological models and help for the conservation of plant-pollinators.

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Table 1. All abiotic variables considered for this study with their names, description, units and whether they were used for downstream analyses (= Yes) or removed because of high collinearity with others (= No).

Variable name	Description	Units	Retained
Bio1	Annual Mean Temperature	°C	Yes
Bio2	Mean Diurnal Range	°C	Yes
Bio3	Isothermality	°C	Yes
Bio4	Temperature Seasonality	°C	No
Bio5	Max Temperature of Warmest Month	°C	Yes
Bio6	Min Temperature of Coldest Month	°C	No
Bio7	Temperature Annual Range	°C	No
Bio8	Mean Temperature of Wettest Quarter	°C	Yes
Bio9	Mean Temperature of Driest Quarter	°C	Yes
Bio10	Mean Temperature of Warmest Quarter	°C	No
Bio11	Mean Temperature of Coldest Quarter	°C	No
Bio12	Annual Precipitation	mm	Yes
Bio13	Precipitation of Wettest Month	mm	No
Bio14	Precipitation of Driest Month	mm	Yes
Bio15	Precipitation Seasonality	mm	No
Bio16	Precipitation of Wettest Quarter	mm	No
Bio17	Precipitation of Driest Quarter	mm	No
Bio18	Precipitation of Warmest Quarter	mm	No
Bio19	Precipitation of Coldest Quarter	mm	No
Altitude	Height	m	No
ACDWRB	Grade of a sub-soil being acid	grade	No
BDRICM	Depth to bedrock	cm	Yes
BDRLOG	Probability of occurrence of R horizon	percentage	No
BLDFIE	Bulk density	kg/m^3	Yes
CECSOL	Cation Exchange Capacity of soil	cmolc/kg	Yes
CLYPPT	Weight percentage of the clay particles	percentage	Yes
CRFVOL	Volumetric percentage of coarse fragments	percentage	No
OCDENS	Soil organic carbon density	kg/m^3	No
OCSTHA	Soil organic carbon stock	ton/ha	No
ORCDRC	Soil organic carbon content	permille	Yes
PHIHOX	pH index measured in water solution	pH	No
PHIKCL	pH index measured in KCl solution	pH	No
SLTPPT	Weight percentage of the silt particles	percentage	Yes
SNDPPT	Weight percentage of the sand particles	percentage	Yes

Table 2. Distribution models evaluation statistics (successful runs, AUC and TSS), for each model used in the study.

Model	Successful runs	AUC	TSS
GLM	100%	0.89	0.71
GAM	100%	0.89	0.67
GBM	100%	0.85	0.64
SVM	100%	0.89	0.64
Random Forest	100%	0.94	0.75
MARS	100%	0.89	0.68
MAXENT	100%	0.91	0.70

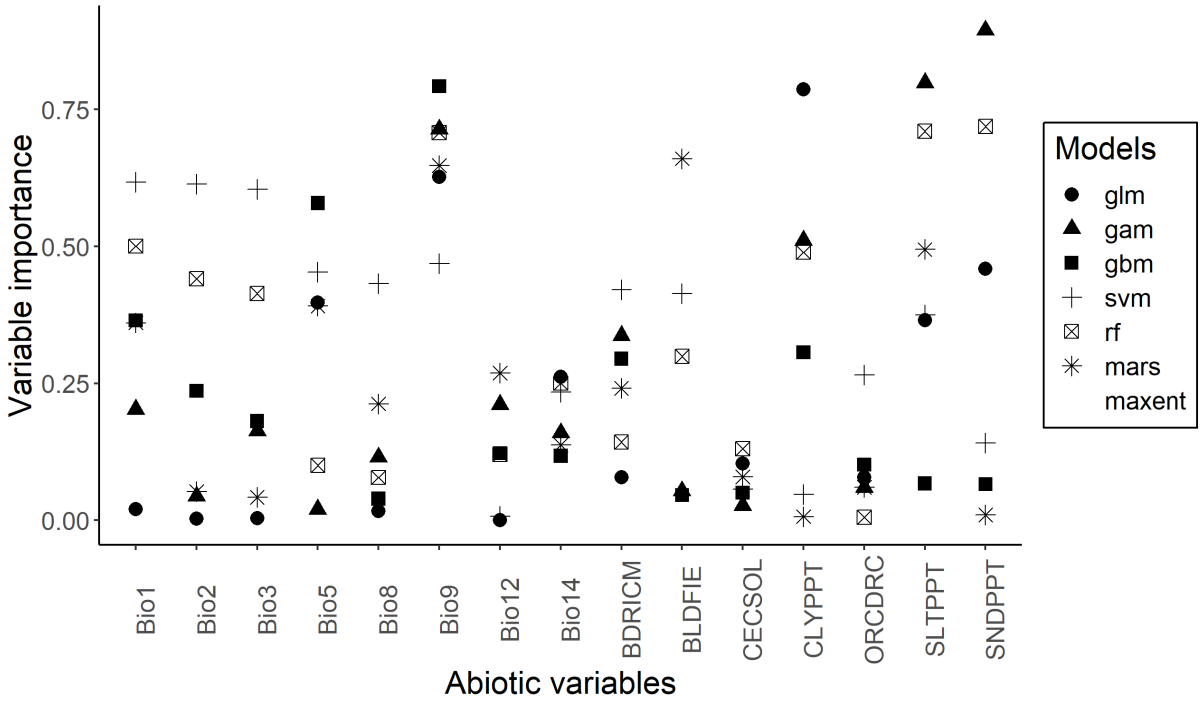


Figure 1. Variable importance for each model. Variables retained for the species distribution modeling are on the x axis. Names correspond to variables described in Table 1. Shapes represent the models used.

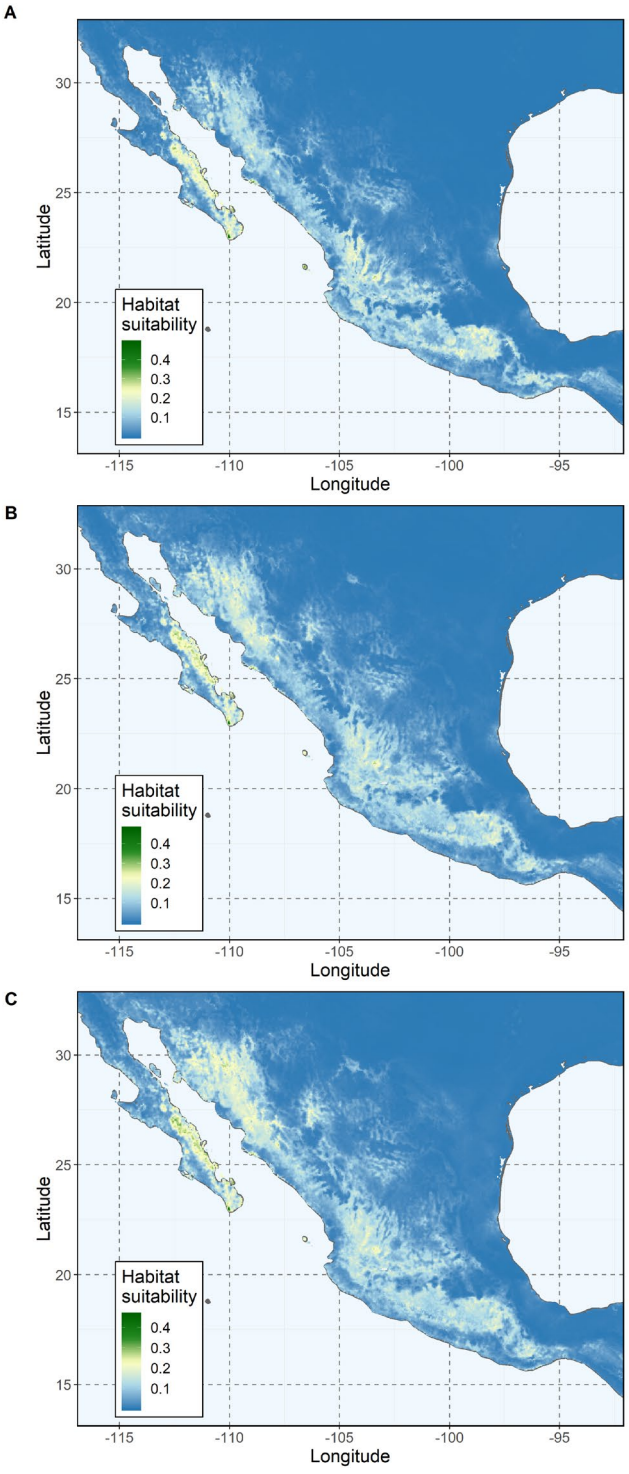


Figure 2. Difference in climatic averages between current and future (FUTURE ENSEMBLE averages). A) Difference in temperature (Bio1) for concentration scenario RCP 4.5; B) difference in precipitation (Bio12) for concentration scenario RCP 4.5; C) difference in temperature (Bio1) for concentration scenario RCP 8.5 and D) difference in precipitation (Bio12) for concentration scenario RCP 8.5.

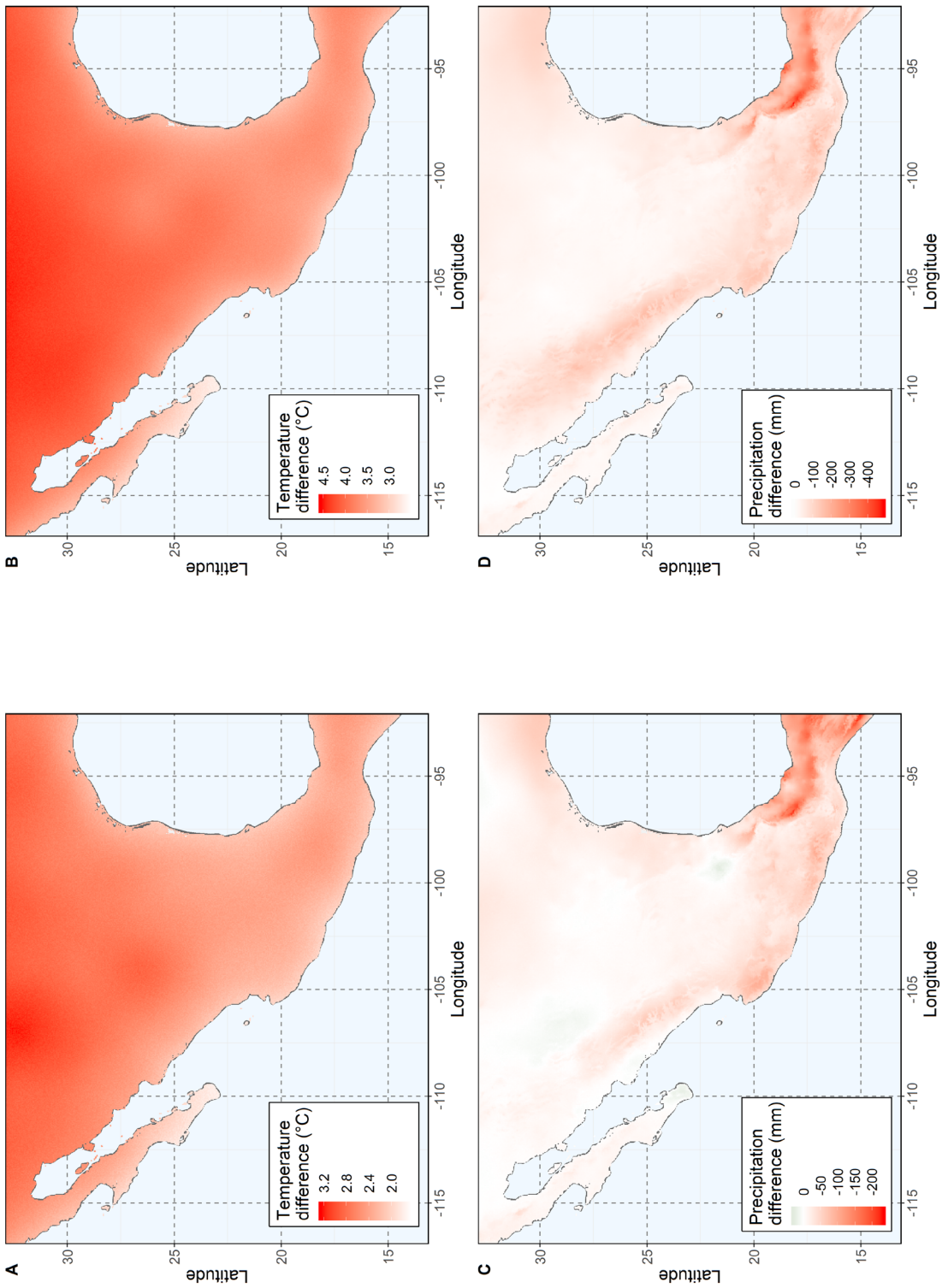


Figure 3. Difference in climatic averages between current and future (FUTURE ENSEMBLE averages). A) Difference in temperature (Bio1) for concentration scenario RCP 4.5; B) difference in precipitation (Bio12) for concentration scenario RCP 4.5; C) difference in temperature (Bio1) for concentration scenario RCP 8.5 and D) difference in precipitation (Bio12) for concentration scenario RCP 8.5.

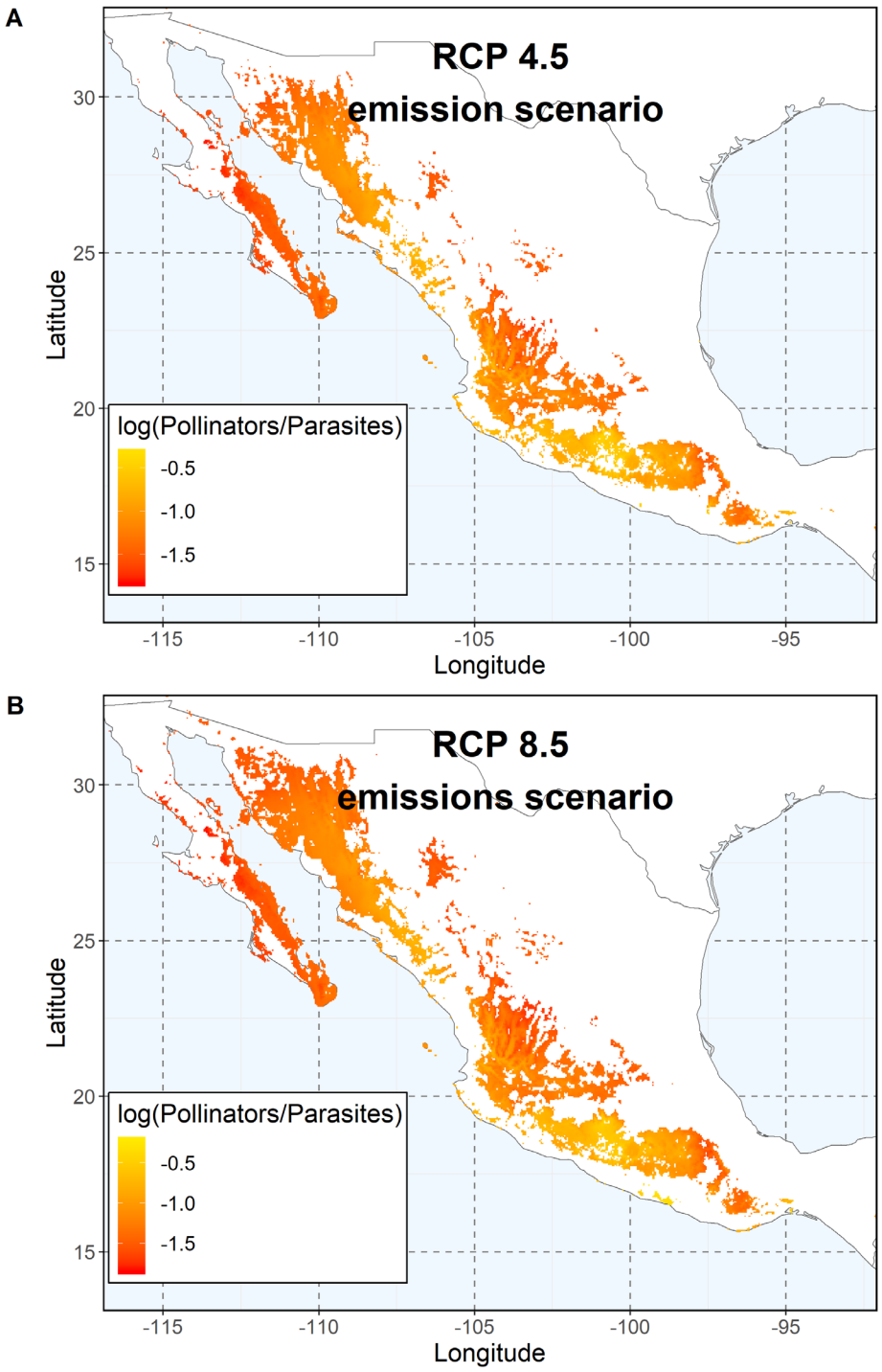


Figure 4. Wasp community dynamic forecast into the future for RCP scenario 4.5 (moderate concentration) and 8.5 (high concentration) in 2070. Colors show the proportion of pollinator relative to non-pollinating fig wasps projected on the future potential distribution of the host tree (i.e., future habitat suitability), associated with future climate temperature and precipitation. Colors are: green to dark green = locations associated with higher production of pollinator; white = equal proportion of pollinators and non-pollinating fig wasps; yellow to red: locations associated with higher production of non-pollinating fig wasps.

CHAPTER 6. GENERAL CONCLUSIONS

The goals motivating the work presented in this dissertation were to highlight the history, the contemporary ecology, and forecast into the future aspects of a fig and its highly-specialized insect community in a geographical context. The *Ficus petiolaris*–pollinator–antagonist system provided a very interesting but rather challenging biological model to work with. Indeed, one characteristic of this system is the extreme variation in its phenology and associated fig wasp community through space and time. Within this "noise", however, I was able to identify ecological factors significantly influencing dynamics in pollinator and antagonist community composition associations, thereby placing insights into fig–insect interactions within the broader community context in which it should be studied`.

In **Chapter 2**, I combined analyses of genomic variation in *F. petiolaris* across its geographical range with historical distribution modeling strengthened our knowledge of the effects of deep- and shallow-time climatic and geological events on its historical demography. These findings confirm similar conclusions drawn from observations made in other organisms, specifically that plants, especially frost-intolerant, have seen their ranges moving towards the equator during late Pleistocene climate oscillations, and then increasing towards the poles during the Holocene. A result of this range shifting is a contemporary genetic landscape with greater genetic diversity in more equatorial ancestral or refugial populations, and lesser genetic diversity in newly colonized, more polar populations. In *F. petiolaris*, we also found strong structure among three regions (Baja California, central and northern western Mexico, and Oaxaca), suggesting sources of vicariance between regional populations which have persisted since before the last glacial maxima. This isolation represents potential for local adaptation and subsequent speciation. This new understanding of the phylogeographic history of *F. petiolaris* will be

foundational for future, comparative phylogeographic studies aimed at revealing the evolutionary histories of co-association between this species and its insect symbionts. Remaining to be addressed in any fig system is the extent to which plant, pollinator, non-pollinator fig wasps, and other antagonists, share similar or divergent histories and genetic structures, and if genetic divergence on the landscape is aligned so as to ultimately supports cospeciation.

Chapter 3 highlighted the importance of studying ecological processes from a spatial and temporal perspective. The *F. petiolaris*–pollinator–antagonist system shows a high degree of variation in many aspects of its biology, making it challenging to find significant associations between the predictor and response variables tested. Owing to the large sample sizes obtained, however, this study allowed me to confirm a long-held hypothesis that was never tested, which is the negative effect of within-tree asynchrony on the fig-fig wasp mutualism. Among other significant findings, were the differential effects of temperature and precipitation on individual was species, which supported the hypothesis that pollinating wasp are protected within their syconia and so less impacted by extreme weather than no-pollinator wasps. My community level approach of investigating multiple species with respect to multiple variables allowed me to test a number hypotheses that had not previously been tested together, and to obtain a more comprehensive understanding of local and landscape variation in fig wasp community dynamics.

My third study presented in **Chapter 4** quantified and investigated the causes of damage to fig fruit (including developing seeds and fig wasps) by lepidopteran larvae. Interestingly, this clade of fig predator has been observed in many Neotropical *Ficus* systems, frequently causing substantial damage to fig crops, and yet remains virtually unknown. My molecular work allowed me identifying the caterpillar associated with *F. petiolaris* as *Omiodes stigmosalis* and to identify the ecological factors influencing its damage to fig crops. I found the pattern of reproduction

characteristic of most fig species – large and highly synchronous fruit crops – to be associated with larger *O. stigmosalis* damage in *F. petiolaris*, whereas smaller, more asynchronous crops tended to limit the caterpillar damage. This suggest that small and asynchronous crops which are often observed in *F. petiolaris* may potentially be an adaption to avoid fig predation.

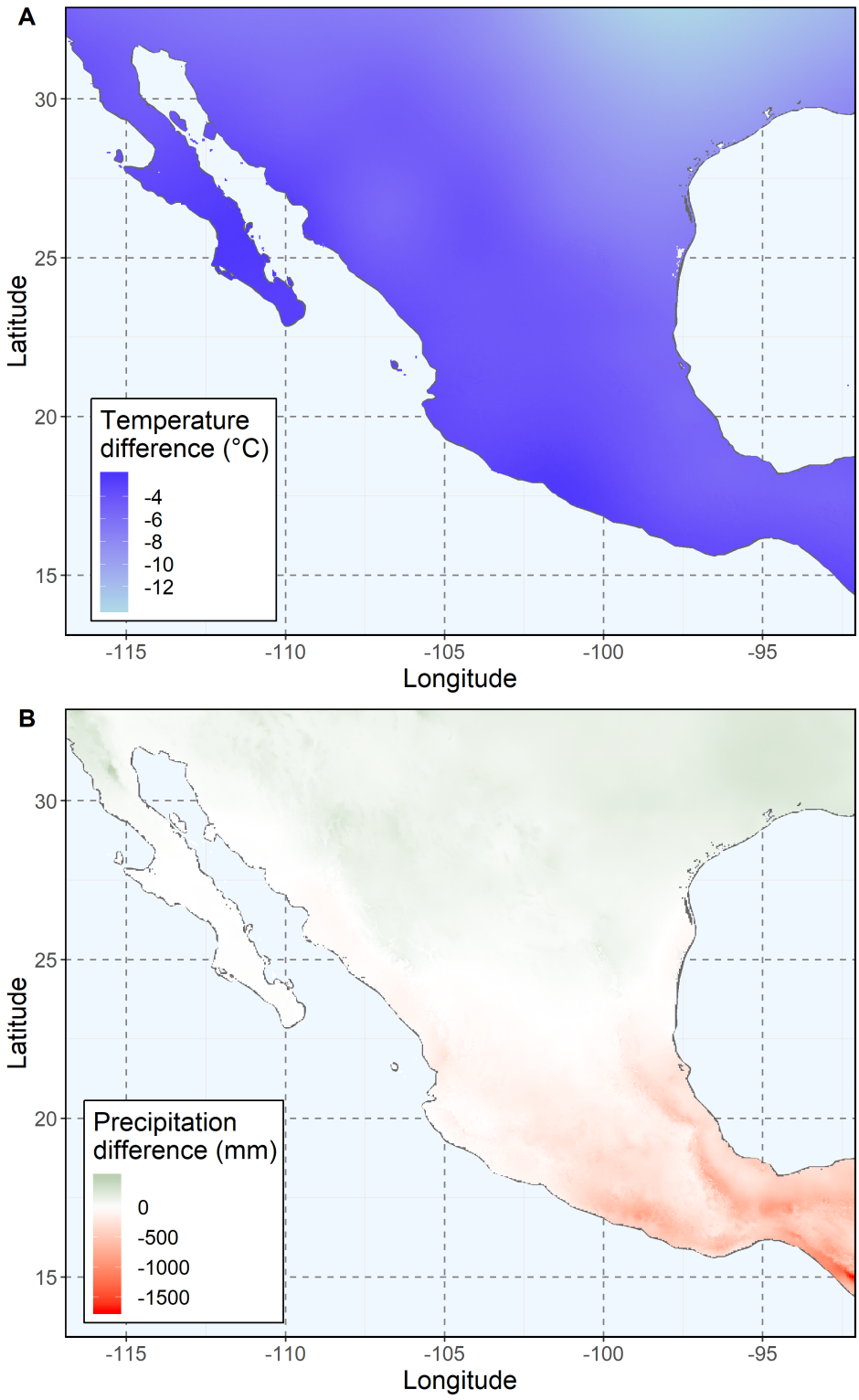
Finally, in **Chapter 5**, I projected the interaction between *F. petiolaris*, its pollinator, and the parasites into the near future under a predicted model of climate change. This work wouldn't have been possible without the ecological modeling work presented in Chapter 2. Here I projected in space and in time the expected *F. petiolaris* pollinator-parasite for the year 2070 using two greenhouse gas concentration scenarios by combining results from multiple ecological models with different species distribution models. The results suggest a future that will benefit the parasites over the pollinators which could have dramatic consequences on the mutualism by reducing the fitness of both pollinator and host plant. The approach I developed allows visualizing expected variation in the fig-pollinator-non-pollinator interaction in the future, and predicting locations on the landscape where mutualism fitness will increase or decrease depending on climate change effects on pollinators verses non-pollinators. I believe that this method has broad application for future research investigating plant-pollinator conservation.

The work presented in this dissertation constitutes materials which strengthen our knowledge of the *F. petiolaris* system, and of fig-fig wasp mutualisms and plant-pollinator interactions in general. Even if many caveats remain, my approach of studying the system holistically, in a community context and from a spatial and temporal perspective, has allowed me to gain a deeper and more comprehensive understanding of the biology of figs and associated insects, and to more fully recognize its value as biological model of mutualistic and antagonistic interactions.

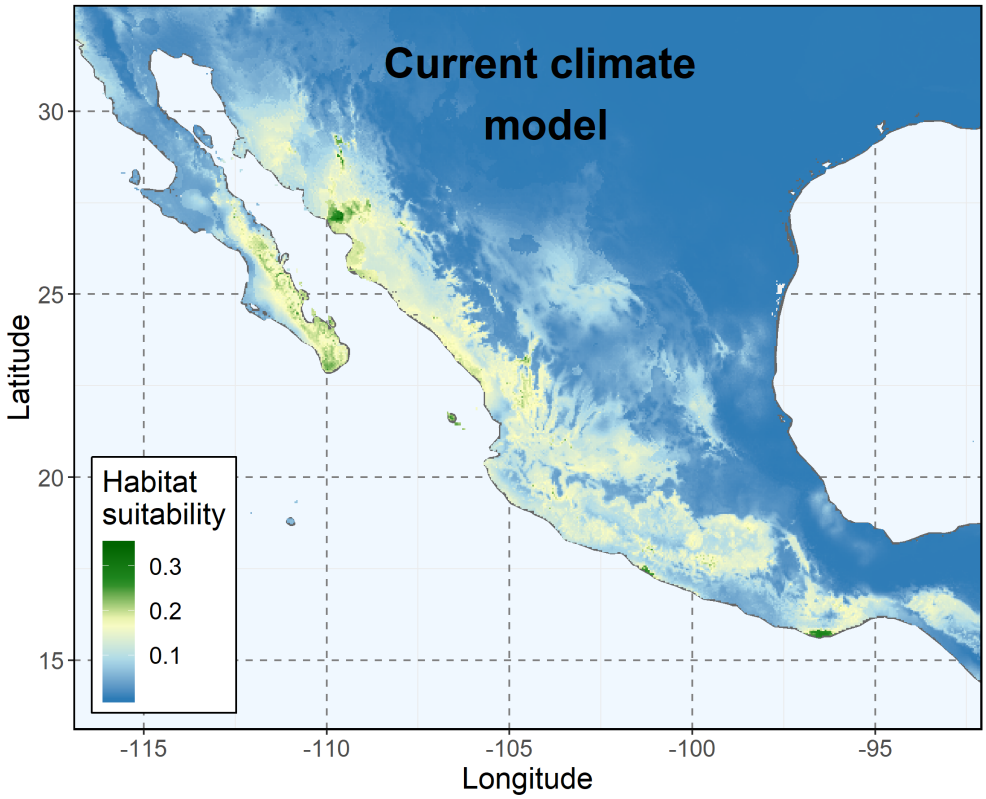
APPENDIX A. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 2

Supplemental Table 1. Evaluation statistics for the habitat suitability models used for this study. GLM = generalized linear model; GAM = generalized additive model; GBM = gradient boosting machine; SVM = support-vector networks; Random Forest = random decision forests; MARS = Multivariate adaptive regression splines; MAXEN = maximum entropy models. The models were evaluated with partitioning of the complete occurrence and pseudo-absence data set.

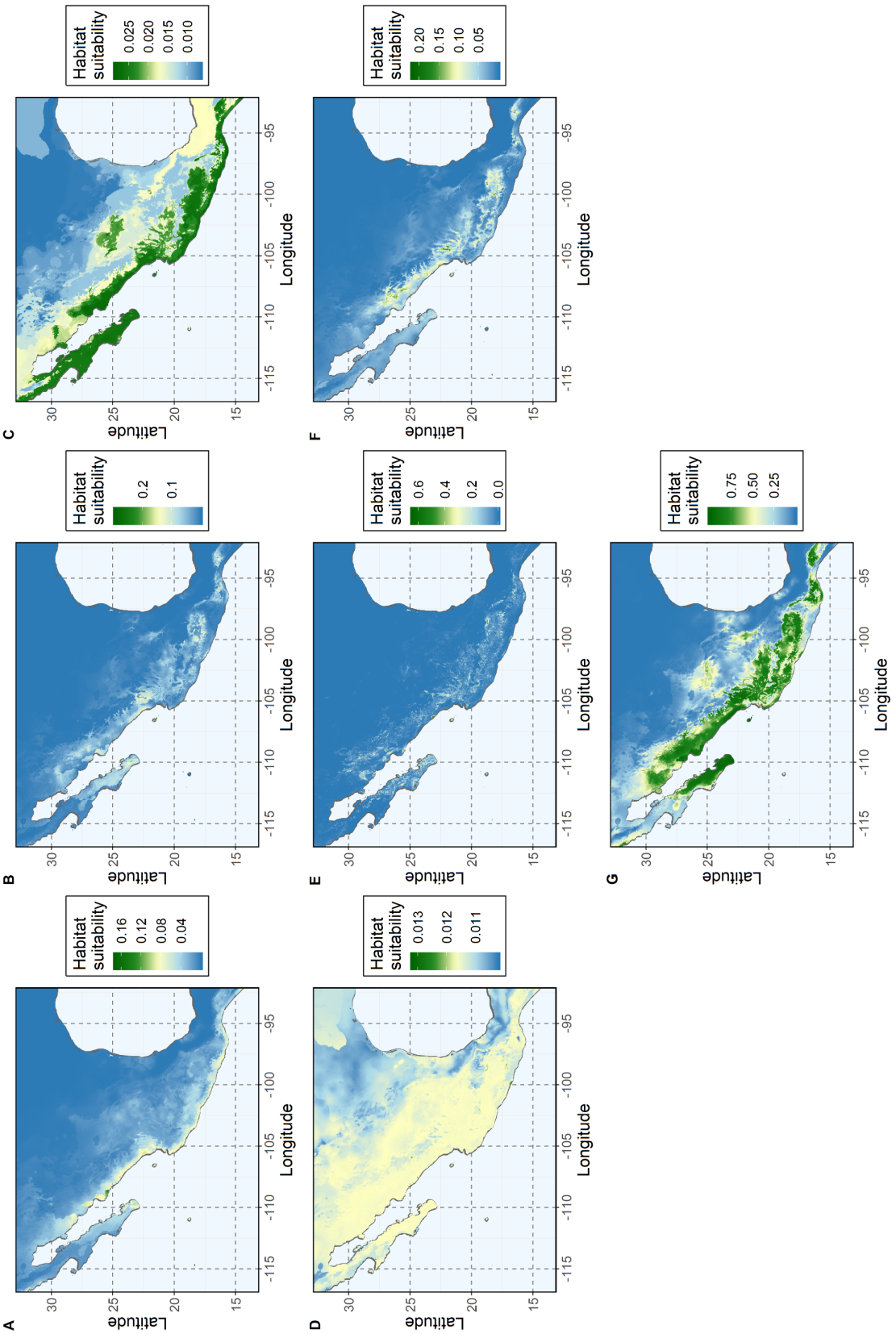
Model	Successful runs	AUC	TSS
GLM	100%	0.82	0.55
GAM	100%	0.87	0.65
GBM	100%	0.80	0.51
SVM	100%	0.84	0.62
Random Forests	100%	0.92	0.73
MARS	100%	0.84	0.61
MAXENT	100%	0.87	0.65



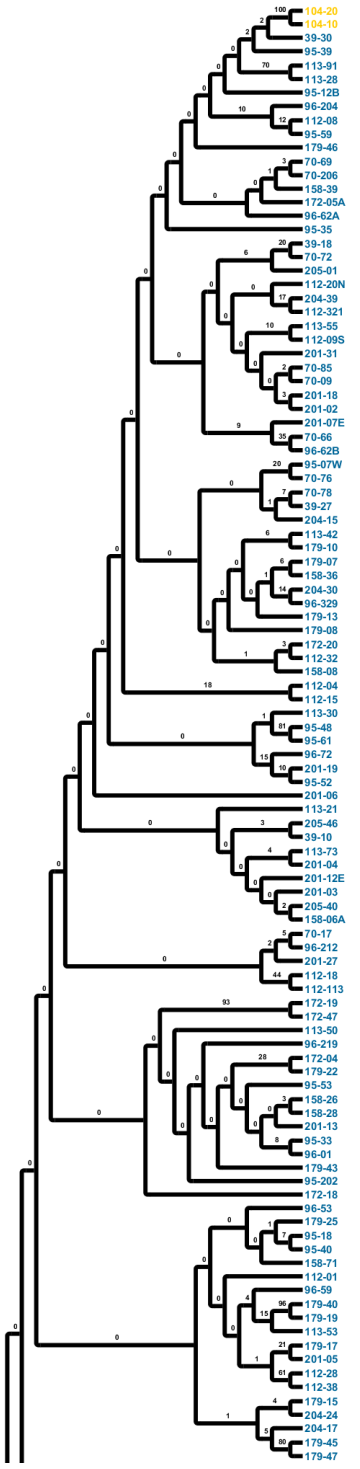
Supplemental Figure 1. Temperature (A) and precipitation (B) differences between LGM and current climatic conditions. Values represent difference between past climatic conditions relative to the current ones.



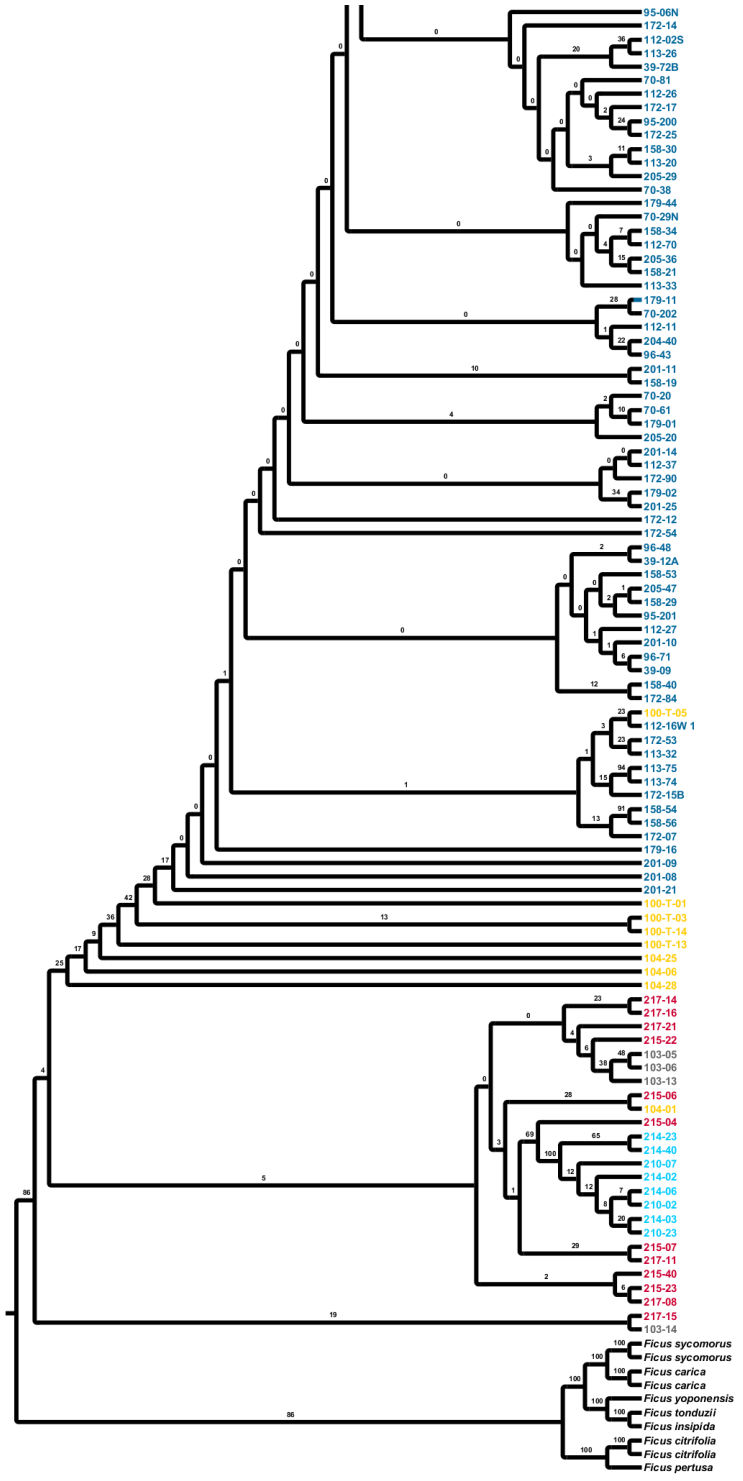
Supplemental Figure 2. Habitat suitability for *F. petiolaris* prediction across Mexico with the current global climatic conditions (worldclim data). 100% of the occurrence data are within suitability >0. The prediction was modeled with a global model combining all the models trained with current climate data, weighted by their TSS value.



Supplemental Figure 3. All projected habitat suitabilities across Mexico for current bioclimatic conditions for seven models. A = GLM, B = GAM, C = GBM, D = SWM, E = Random Forest, F = MARS, G = MAXENT.



Supplemental Figure 4. Maximum likelihood phylogenetic tree of *F. petiolaris*' individuals with uncollapsed branches. Node values represent bootstrap support for 100 replicates. Colors represent identified phylogroups, outgroups are labels colored in black.



Supplemental Figure 4. Continued.

Supplemental Table 1. Summary data across sites and seasons for collected *F. petiolaris* syconia and counts of pollinating and non-pollinating fig wasps reared from them. Total wasps include unidentified damaged wasps. NPFW: non-pollinating fig wasps; SD: standard deviation; NA: missing data.

Collecting trip	Sites	D phase syconia collected	Syconia volume (mm3)		Foundresses		Nematode infection	*Pegoscapus sp.*			*Idarnes flavicolis* sp. 1		
		N	Mean	SD	Mean	SD	Proportion	Sum	Mean	SD	Sum	Mean	SD
Fall 2012	158	72	1235	573.04	1.31	0.71	34.80%	87	1.21	2.34	334	4.64	7.34
	172	66	1081	681.63	1.61	0.89	75.80%	257	3.89	5.20	483	7.32	9.27
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	99	2140	661.73	1.29	0.93	77.10%	1324	13.37	24.37	1175	11.87	14.89
	95	104	1184	603.34	1.67	1.05	63.00%	2750	26.44	33.93	1013	9.74	11.49
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	105	2631	1099.74	1.72	1.31	50.00%	4060	38.67	44.20	940	8.95	10.69
	96	100	1331	649.34	1.44	0.84	38.40%	4361	43.61	37.68	1204	12.04	9.05
	70	102	1565	708.23	1.52	0.87	40.60%	1938	19.00	21.91	805	7.89	9.24
Spring 2013	158	85	1631	581.87	1.04	0.57	31.30%	1600	18.82	28.40	1685	19.82	18.34
	172	106	962	687.79	1.34	0.64	23.50%	951	8.97	20.54	3350	31.60	29.80
	112	96	2254	846.72	1.51	1.20	45.70%	2806	29.23	26.82	2537	26.43	22.54
	113	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	95	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	13	1119	148.03	3.46	2.88	61.50%	18	1.38	2.63	7	0.54	0.78
	96	99	2556	1231.47	1.87	1.50	41.70%	8970	90.61	107.25	691	6.98	16.54
	70	61	2498	900.84	1.21	0.71	21.30%	2077	34.05	42.71	614	10.07	13.65
Fall 2013	158	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	172	78	3092	1400.82	1.35	0.74	16.90%	8756	112.26	81.78	752	9.64	11.71
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	57	3164	1303.28	1.23	0.50	29.40%	5528	96.98	68.40	502	8.81	15.90
	95	59	1442	463.95	0.56	0.65	20.30%	384	6.51	14.33	698	11.83	10.43
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	96	100	1635	1072.56	0.91	0.79	24.50%	2029	20.29	34.61	2241	22.41	18.58
	70	100	2337	973.36	1.14	0.55	40.80%	8493	84.93	75.95	1913	19.13	23.15
Spring 2014	158	24	2273	844.83	0.75	0.44	12.50%	587	24.46	40.25	263	10.96	16.36
	172	93	1990	560.92	1.49	0.74	37.80%	2779	29.88	29.43	1034	11.12	12.43
	112	99	1117	615.80	1.37	1.02	30.90%	1986	20.06	23.67	569	5.75	7.77
	113	102	1204	364.31	0.96	0.42	27.50%	395	3.87	4.43	124	1.22	2.65
	95	114	3047	913.06	1.18	0.55	7.90%	11016	96.63	65.86	2408	21.12	19.86
	179	118	942	359.40	1.00	0.50	0.00%	4515	38.26	40.98	1892	16.03	16.92
	201	112	3358	1436.49	0.69	0.55	15.20%	2055	18.35	35.38	6200	55.36	36.58
	96	103	1863	684.83	1.63	1.00	40.20%	2143	20.81	27.59	1713	16.63	27.20
	70	100	2121	1207.34	1.74	0.88	26.30%	6637	66.37	65.38	2921	29.21	28.03

Supplemental Table 1. Continued

Collecting trip	Sites	*Idarnes carme* sp. 1			*Idarnes carme* sp. 2			*Heterandrium* sp. 1			*Heterandrium* sp. 2		
		Sum	Mean	SD	Sum	Mean	SD	Sum	Mean	SD	Sum	Mean	SD
Fall 2012	158	348	4.83	7.63	442	6.14	7.74	32	0.44	1.29	48	0.67	1.26
	172	515	7.80	7.95	779	11.80	10.64	228	3.45	5.68	171	2.59	3.67
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	723	7.30	8.60	2454	24.79	17.34	461	4.66	7.03	289	2.92	4.01
	95	391	3.76	6.61	914	8.79	9.72	840	8.08	10.60	194	1.87	3.45
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	233	2.22	3.48	930	8.86	9.40	254	2.42	3.98	99	0.94	2.39
	96	189	1.89	3.84	164	1.64	3.14	390	3.90	4.85	124	1.24	2.12
	70	1005	9.85	9.37	670	6.57	7.70	354	3.47	5.18	205	2.01	2.94
Spring 2013	158	189	2.22	3.37	680	8.00	9.03	186	2.19	5.07	42	0.49	1.29
	172	234	2.21	5.03	797	7.52	10.06	258	2.43	6.91	78	0.74	1.88
	112	239	2.49	4.95	262	2.73	5.21	287	2.99	5.60	350	3.65	4.70
	113	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	95	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	35	2.69	3.92	119	9.15	8.07	10	0.77	1.17	17	1.31	2.50
	96	702	7.09	9.03	992	10.02	12.37	73	0.74	2.14	145	1.46	2.76
	70	455	7.46	10.44	65	1.07	2.82	149	2.44	6.57	50	0.82	1.69
Fall 2013	158	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	172	579	7.42	13.71	1121	14.37	16.14	135	1.73	3.35	144	1.85	4.40
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	161	2.82	5.66	314	5.51	10.46	107	1.88	3.74	107	1.88	2.82
	95	423	7.17	13.40	112	1.90	5.23	301	5.10	7.08	141	2.39	3.49
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	96	151	1.51	4.07	117	1.17	2.98	1238	12.38	15.68	80	0.80	1.99
	70	618	6.18	10.93	429	4.29	8.60	526	5.26	9.12	160	1.60	4.33
Spring 2014	158	22	0.92	3.02	106	4.42	9.86	20	0.83	1.79	38	1.58	3.96
	172	1001	10.76	12.80	2322	24.97	21.13	643	6.91	8.23	553	5.95	6.38
	112	420	4.24	5.91	1992	20.12	11.96	436	4.40	6.53	676	6.83	8.39
	113	384	3.76	4.61	2256	22.12	11.66	237	2.32	2.99	135	1.32	1.82
	95	497	4.36	7.08	569	4.99	8.93	50	0.44	1.43	91	0.80	2.72
	179	551	4.67	9.41	785	6.65	12.45	69	0.58	1.84	152	1.29	4.31
	201	47	0.42	1.87	225	2.01	4.70	589	5.26	7.92	22	0.20	0.66
	96	1232	11.96	14.67	2311	22.44	19.37	258	2.50	4.10	216	2.10	3.50
	70	1898	18.98	19.32	314	3.14	7.15	225	2.25	5.63	964	9.64	9.20

Supplemental Table 1. Continued

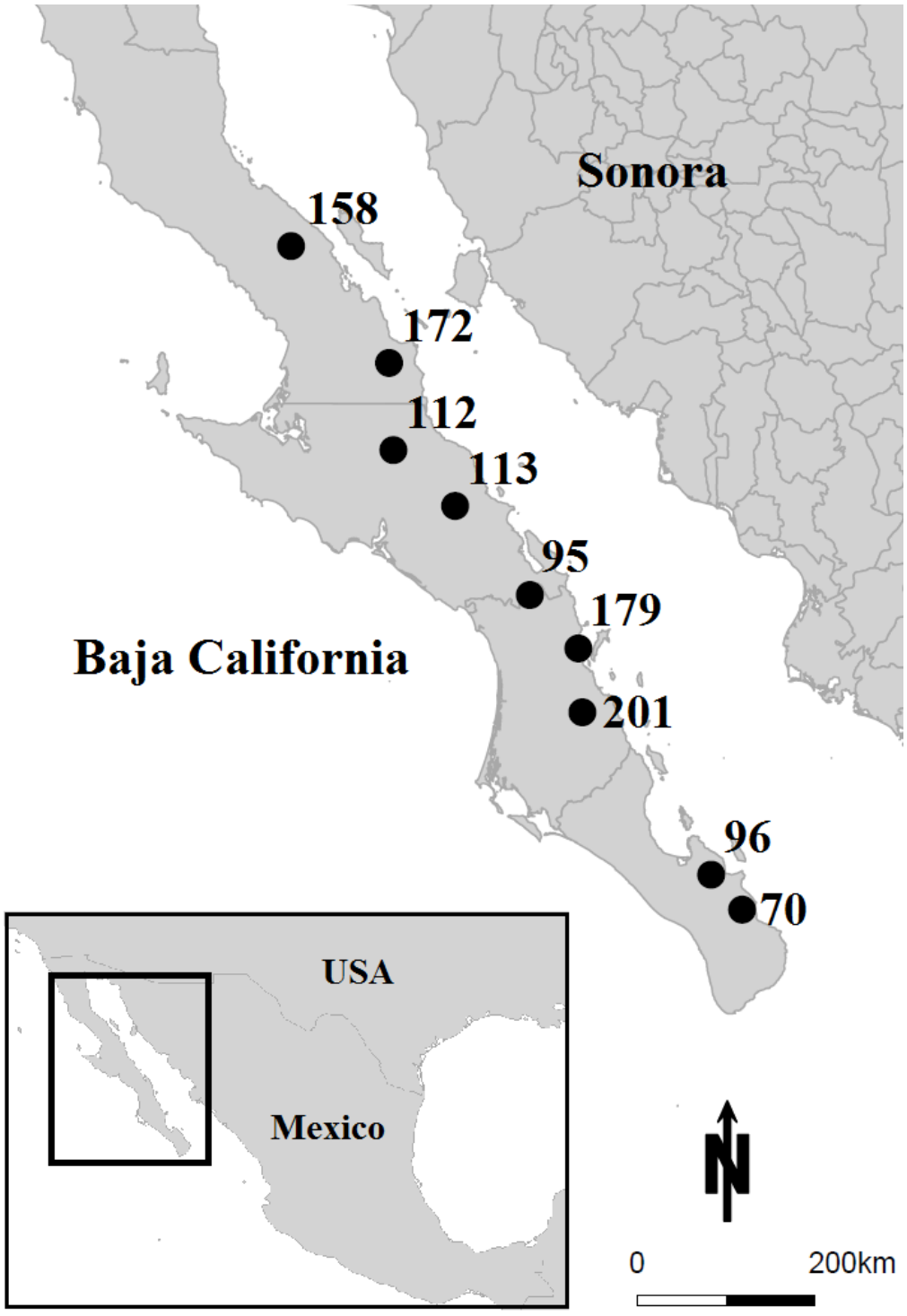
Collecting trip	Sites	*Ficicola* sp.			*Physothorax* sp.			*Sycophila* sp.			Total wasps	NPFW
		Sum	Mean	SD	Sum	Mean	SD	Sum	Mean	SD	Sum	Proportion
Fall 2012	158	3	0.04	0.20	167	2.32	3.64	0	0.00	0.00	3161	97%
	172	13	0.20	0.56	9	0.14	0.52	0	0.00	0.00	3520	93%
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	18	0.18	0.73	51	0.52	1.09	4	0.04	0.24	8268	84%
	95	20	0.19	0.52	213	2.05	3.03	2	0.02	0.14	7417	63%
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	89	0.85	1.89	369	3.51	5.79	19	0.18	1.12	7868	48%
	96	19	0.19	0.53	41	0.41	1.83	4	0.04	0.24	7090	38%
	70	33	0.32	0.96	29	0.28	0.87	2	0.02	0.14	6448	70%
Spring 2013	158	20	0.24	0.95	2	0.02	0.22	0	0.00	0.00	5628	72%
	172	22	0.21	1.08	13	0.12	0.63	26	0.25	2.53	7702	88%
	112	131	1.36	4.90	46	0.48	1.79	16	0.17	1.34	8118	65%
	113	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	95	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	5	0.38	1.12	20	1.54	2.60	5	0.38	0.65	328	95%
	96	16	0.16	0.60	72	0.73	2.46	8	0.08	0.47	13749	35%
	70	87	1.43	3.55	101	1.66	3.63	8	0.13	0.53	4268	51%
Fall 2013	158	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	172	164	2.10	3.56	437	5.60	6.97	3	0.04	0.25	14043	38%
	112	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	113	84	1.47	4.46	36	0.63	1.51	3	0.05	0.23	7124	22%
	95	60	1.02	2.14	191	3.24	3.46	3	0.05	0.39	2702	86%
	179	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	201	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	96	125	1.25	1.88	183	1.83	2.22	2	0.02	0.14	6918	71%
	70	123	1.23	2.14	192	1.92	3.65	26	0.26	0.76	13196	36%
Spring 2014	158	14	0.58	2.30	13	0.54	1.10	2	0.08	0.41	1213	51%
	172	74	0.80	1.39	90	0.97	1.87	1	0.01	0.10	10911	74%
	112	56	0.57	1.10	49	0.49	1.70	0	0.00	0.00	7920	75%
	113	164	1.61	1.89	424	4.16	3.60	9	0.09	0.29	5264	92%
	95	147	1.29	2.48	131	1.15	2.45	0	0.00	0.00	15936	31%
	179	110	0.93	2.19	9	0.08	0.57	4	0.03	0.29	9262	51%
	201	91	0.81	1.96	10	0.09	0.51	2	0.02	0.19	11805	83%
	96	67	0.65	1.93	153	1.49	2.96	17	0.17	0.49	10977	80%
	70	24	0.24	0.77	81	0.81	1.90	3	0.03	0.30	15128	56%

Supplemental Table 2. Summary statistics across sites and seasons of *F. petiolaris* tree-level (Reproduction, Asynchrony, and Syconium landscape) and site-level (Temperature and Precipitation) variables. SD: standard deviation, NA: missing data.

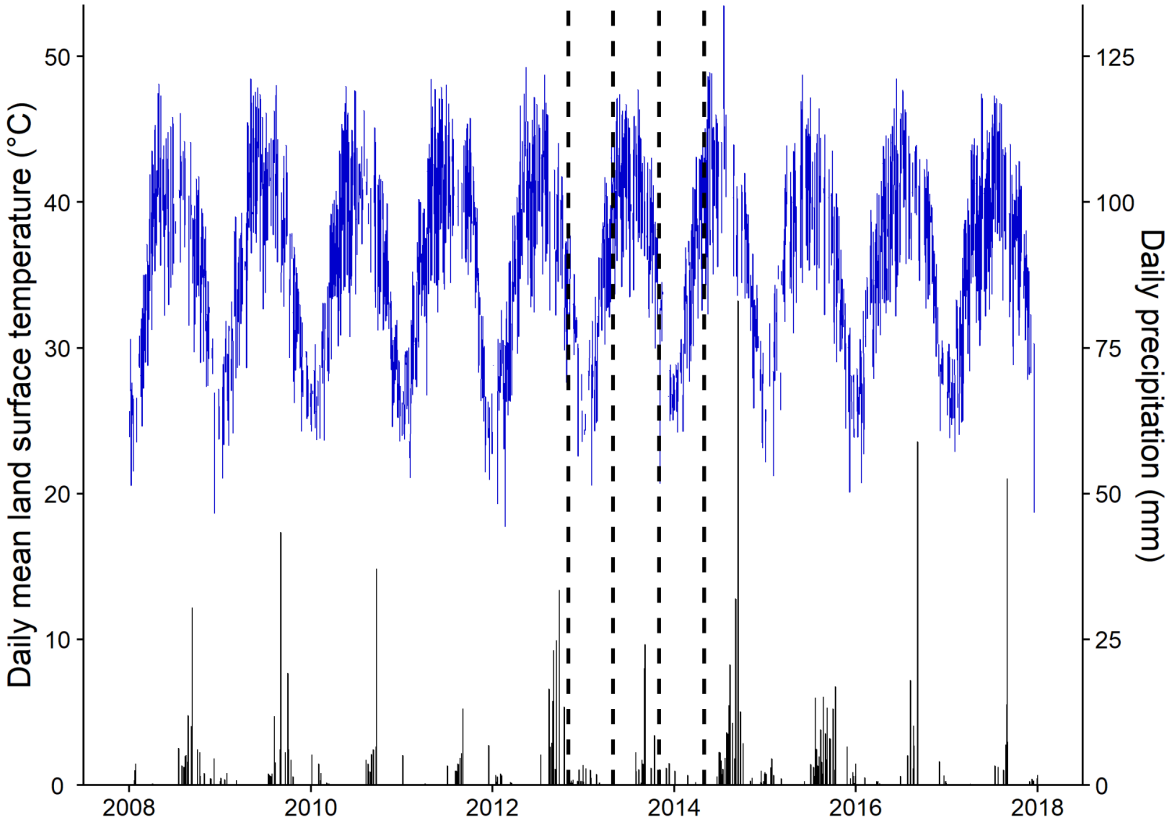
Collection trip	Sites	Reproduction (%)		Asynchrony		Syconium landscape		Land surface temperature (°C)	Precipitation (mm^3)
		Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	Sum
Fall 2012	158	NA	NA	2.52	1.06	NA	NA	36.08	25.31
	172	0.02	0.09	2.16	0.87	17.60	37.38	34.78	24.25
	112	0.08	0.21	1.71	0.88	3.36	13.32	31.93	22.76
	113	0.10	0.24	2.56	0.98	478.05	1322.17	32.22	19.13
	95	0.10	0.25	2.21	0.66	533.94	923.80	34.09	42.22
	179	0.02	0.07	2.00	0.63	3.19	10.62	35.75	60.80
	201	NA	NA	2.93	1.30	0.00	0.00	35.04	129.62
	96	0.09	0.21	2.03	1.08	8.87	55.49	38.57	93.76
	70	0.05	0.18	2.68	0.89	71.62	282.09	33.40	111.45
Summer 2013	158	0.20	0.35	2.28	0.96	197.55	182.79	34.12	17.20
	172	0.09	0.20	2.67	1.07	196.15	139.21	35.62	1.35
	112	0.11	0.22	1.60	0.83	9.81	17.46	33.86	1.00
	113	0.05	0.16	2.23	1.20	319.14	444.48	37.10	3.58
	95	0.16	0.31	1.63	0.71	381.96	446.09	40.04	0.25
	179	NA	NA	NA	NA	NA	NA	NA	NA
	201	NA	NA	1.98	0.99	NA	NA	39.06	0.20
	96	0.12	0.27	3.23	0.77	48.86	261.74	42.60	0.00
	70	0.27	0.28	2.16	0.88	460.63	516.69	39.24	0.00
Fall 2013	158	0.01	0.03	1.51	0.73	0.91	0.73	36.02	37.19
	172	0.00	0.00	2.13	0.87	0.12	0.08	42.47	43.32
	112	0.13	0.26	1.78	0.64	24.81	44.37	38.09	21.25
	113	0.06	0.19	1.82	0.91	67.75	206.73	35.23	22.15
	95	0.14	0.25	2.33	0.77	252.61	479.60	33.21	22.56
	179	0.04	0.13	1.29	0.43	7.69	13.44	NA	NA
	201	0.04	0.15	1.72	0.71	42.11	178.60	NA	NA
	96	0.03	0.12	2.42	0.90	61.11	209.63	36.71	22.39
	70	0.10	0.23	2.43	0.80	271.58	430.62	32.98	96.95
Summer 2014	158	0.18	0.34	1.56	0.53	226.96	172.12	38.96	0.00
	172	0.12	0.28	1.79	0.87	260.99	293.98	35.14	0.00
	112	0.28	0.39	1.59	0.69	156.60	448.70	34.04	1.96
	113	0.14	0.27	2.08	0.86	1419.10	2497.05	38.14	4.70
	95	0.07	0.21	2.00	0.77	169.43	150.42	41.26	0.25
	179	0.12	0.26	2.12	0.93	31.02	56.16	36.80	0.94
	201	0.26	0.38	2.19	0.94	501.06	1300.69	42.68	0.00
	96	0.06	0.18	2.45	0.99	9.29	26.44	39.47	1.25
	70	0.15	0.26	3.07	1.04	347.99	507.61	40.44	0.00

Supplemental Table 3. Site-level mean and standard deviation of *Ficus petiolaris* crown volume.

	Tree volume (m3)	
Sites	Mean	SD
158	40.06	87.55
172	122.97	253.83
112	92.36	190.41
113	859.77	1266.12
95	650.40	960.14
179	91.38	128.24
201	660.94	735.44
96	238.74	434.98
70	602.23	1033.24



Supplemental Figure 1. Locations of *F. petiolaris*' nine main populations sampled for this study. The numbers represent the site names.



Supplemental Figure 2. Daily average land surface temperature (MODIS MOD11A1) represented by blue lines and precipitation (CHIRPS) represented by black lines, an example with La Paz (Baja California Sur) over one decade. Dashed vertical lines represent the four field seasons of collection for this study (respectively: fall 2012, spring 2013, fall 2013 and spring 2014), and illustrate different climatic conditions at each time.

APPENDIX C. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 4

Supplemental Table 1. Season- and site-level data on the numbers of sampled and damaged figs proportion (with 95% confidence interval).

Season and site	Total fruits sampled	Damaged fruits	Damage proportion [95% CI]
Spring 2013			
Site 158	1140	79	0.07 [0.005, 0.085]
Site 172	840	113	0.13 [0.112, 0.159]
Site 112	960	86	0.09 [0.072, 0.109]
Site 113	1180	470	0.40 [0.370, 0.427]
Site 95	N/A	N/A	N/A
Site 179	N/A	N/A	N/A
Site 201	N/A	N/A	N/A
Site 96	1020	73	0.07 [0.057, 0.089]
Site 70	2220	207	0.09 [0.081, 0.106]
Total	7420	1029	0.14 [0.131, 0.147]
Fall 2013			
Site 158	845	109	0.13 [0.107, 0.153]
Site 172	651	31	0.05 [0.033, 0.067]
Site 112	1018	13	0.01 [0.007, 0.022]
Site 113	1263	82	0.06 [0.052, 0.080]
Site 95	1560	137	0.09 [0.074, 0.103]
Site 179	725	14	0.02 [0.011, 0.032]
Site 201	283	16	0.06 [0.033, 0.090]
Site 96	1430	134	0.09 [0.079, 0.110]
Site 70	2075	66	0.03 [0.025, 0.040]
Total	9850	602	0.06 [0.056, 0.066]
Spring 2014			
Site 158	1155	269	0.23 [0.209, 0.258]
Site 172	855	85	0.10 [0.080, 0.121]
Site 112	2025	27	0.01 [0.009, 0.019]
Site 113	1721	90	0.05 [0.042, 0.064]
Site 95	1545	29	0.02 [0.013, 0.027]
Site 179	960	4	0.00 [0.001, 0.011]
Site 201	930	5	0.01 [0.002, 0.013]
Site 96	2340	9	0.00 [0.002, 0.007]
Site 70	1960	123	0.06 [0.052, 0.074]
Total	13491	658	0.05 [0.045, 0.053]

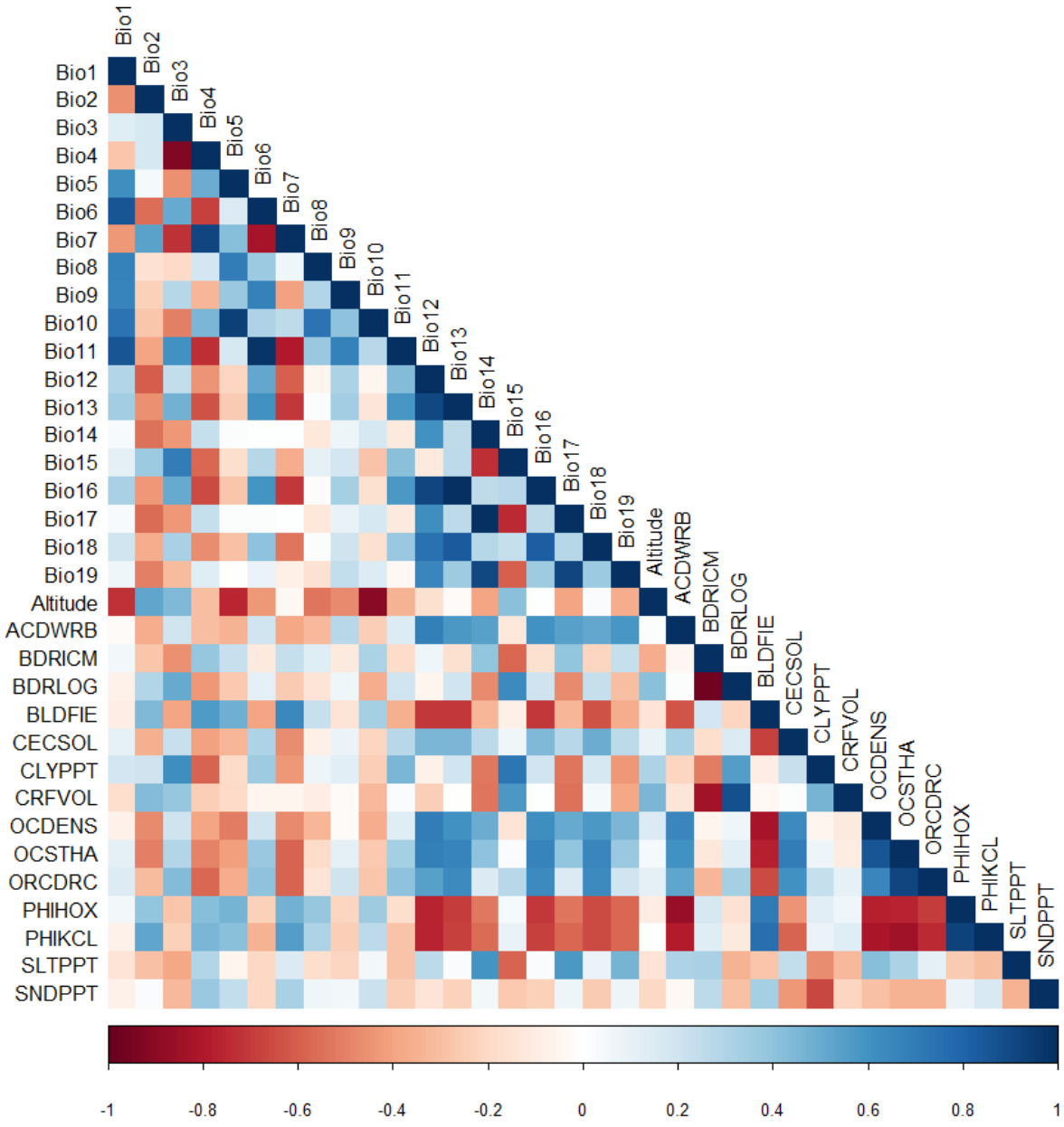
Supplemental Table 2. Season- and site-level data on the proportion of reproductive trees and average within-tree reproductive synchrony/asynchrony.

Season and site	Proportion of reproductive trees	Average synchrony/asynchrony
Spring 2013		
Site 158	0.29 (19/65)	2.28
Site 172	0.33 (14/42)	2.67
Site 112	0.3 (15/50)	1.60
Site 113	0.47 (20/43)	2.23
Site 95	NA	NA
Site 179	NA	NA
Site 201	NA	NA
Site 96	0.31 (18/58)	3.23
Site 70	0.65 (37/57)	2.16
Total	0.39 (123/315)	2.35
Fall 2013		
Site 158	0.25 (18/71)	1.51
Site 172	0.23 (14/61)	2.13
Site 112	0.34 (21/62)	1.78
Site 113	0.37 (24/65)	1.82
Site 95	0.54 (26/48)	2.33
Site 179	0.54 (13/24)	1.29
Site 201	0.41 (7/17)	1.72
Site 96	0.34 (29/85)	2.42
Site 70	0.54 (37/69)	2.16
Total	0.38 (189/497)	2.06
Spring 2014		
Site 158	0.31 (20/64)	1.56
Site 172	0.29 (15/52)	1.79
Site 112	0.68 (41/60)	1.59
Site 113	0.59 (30/51)	2.08
Site 95	0.48 (29/61)	2.00
Site 179	0.61 (19/31)	2.12
Site 201	0.42 (16/38)	2.19
Site 96	0.51 (39/77)	2.45
Site 70	0.53 (34/64)	3.07
Total	0.49 (243/498)	2.15

Supplemental Table 3. Results of spatial autocorrelation analysis of lepidopteran damage of fig fruit by site and season. Bolded rows represent the five site and season combinations in which significant evidence of spatial aggregation of lepidopteran damage was observed at the smallest distance interval. The lone significant negative spatial association is indicated in italics.

Site	Season	Distance interval (m)	No. of pairs	Mean distance (m)	Correlation	*p*-value
70	2013-06	150	62	63.28	-0.14	0.13
70	**2013-11**	**150**	**63**	**69.47**	**0.17**	**0.02**
70	2014-06	150	55	68.54	0.07	0.18
96	2013-06	150	21	82.14	-0.18	0.26
96	2013-11	150	44	83.85	-0.18	0.12
96	2014-06	150	74	85.29	-0.05	0.46
201	2013-11	250	5	135.37	-0.04	0.38
201	2014-06	250	21	136.56	-0.18	0.11
179	2013-11	150	12	63.45	0.22	0.08
179	2014-06	150	23	83.40	-0.15	0.21
95	**2013-11**	**150**	**43**	**62.27**	**0.18**	**0.04**
95	2014-06	150	65	70.06	0.04	0.17
113	2013-06	100	22	63.20	-0.06	0.47
113	2013-11	100	20	58.89	-0.19	0.24
113	**2014-06**	**100**	**44**	**58.86**	**0.24**	**0.03**
112	**2013-06**	**250**	**30**	**147.80**	**0.15**	**0.03**
112	2013-11	250	36	124.56	-0.13	0.30
112	2014-06	250	94	128.26	-0.08	0.27
172	2013-06	100	29	67.82	-0.14	0.37
172	2013-11	100	14	64.19	-0.05	0.46
172	2014-06	100	19	68.21	-0.10	0.47
158	2013-06	50	14	25.84	-0.11	0.48
158	*2013-11*	*50*	*6*	*23.51*	*-0.61*	*0.04*
158	**2014-06**	**50**	**12**	**34.72**	**0.30**	**0.05**

APPENDIX D. ADDITIONAL TABLES AND FIGURES FOR CHAPTER 5



Supplemental Figure 1. Correlation of abiotic variables. Color gradients illustrate correlation levels: blue = positively correlation; red = negative correlation; white = no correlation.